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Biology and Immature Stages of the Bee Tribe Tetrapediini (Hymenoptera: Apidae)

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CONTENTS

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ABSTRACT

Tetrapedia diversipes Klug is herein reported for the first time to be the host of the cleptoparasite *Coelioxoides waltheriae* Ducke. Because these two genera had been previously recognized as sister taxa [A. Roig-Alsina. 1990. *Coelioxoides* Cresson, a parasitic genus of Tetrapediini (Hymenoptera: Apoidea). Journal of the Kansas Entomological Society 63: 279–287], the authors wished to learn to what extent biological information and immature stages reflected this relationship. *Tetrapedia diversipes* normally nests in holes in wood such as old beetle burrows, and it was induced to use trap nests for this study. Many aspects of the nesting behavior of females of this species are described, including the following: diurnal flight period; sleeping habits; nest structure; nest provisioning; egg placement; and sequence of nest construction, provisioning, and oviposition. Eggs produced by this species are categorized as "giant" (K. Iwata and S.F. Sakagami. 1966. Gigantism and dwarfism in bee eggs in relation to the mode of life, with notes on the number of ovarioles. Japanese Journal of Ecology 16: 4–16). Its first instar was discovered to be pharate within the chorion while the following four instars actively feed. Defecation starts early in the last larval stadium. Females use floral oils both in nest construction and in provisioning, and they carry pollen, oil, and soil with their scopae. The biology of *T. diversipes* was compared with that of other species in the genus and then compared with that of other apines that are known to nest in preformed cavities and that provision nests with pollen and floral oils.

The host-nest searching behavior of *Coelioxoides waltheriae* is described. The cleptoparasite introduces its egg into the closed cell of the host shortly after cell closure. This egg is characterized as "small" (Iwata and Sakagami, ibid.) and has a very short incubation period. The highly modified first instar immediately feeds on the host egg and grows remarkably fast on the host yolk. This species has only four instars.

Rates of development of the host and cleptoparasite are compared. Both have four ovarioles per ovary. Eggs, first instars, last larval instars, and pupae of host and cleptoparasite are taxonomically described and compared. In conclusion, the immatures of *Coelioxoides* and *Tetrapedia* are quite distinct from those of other known apids. While these two genera are probably sister genera based on the similarities identified by Roig-Alsina (op. cit.) and by this study, they are quite different from one another based on features of the eggs, first instars, and pupae.

INTRODUCTION

The genus *Coelioxoides* has long been known to be cleptoparasitic because of its anatomical features (see Roig-Alsina, 1990, for enumeration of characters), but its host has remained unknown (Michener et al., 1994), as has other information about its biology. Roig-Alsina (1990), through a careful analysis of its adult anatomical features, assigned it to the Tetrapediini, which before had contained only *Tetrapedia* (Michener and Moure, 1957). The first two authors of the current study discovered that *Tetrapedia diversipes* Klug will utilize trap nests for brood rearing, and the second author (GARM) and later the first author (IAS) found that nests of this bee were parasitized by *Coelioxoides waltheriae* Ducke. These discoveries permit us to describe here the nesting biology of the host, the biology of the cleptoparasite, and the immature stages of both host and cleptoparasite.

This new information in turn allows us to evaluate further the phylogenetic relations of *Tetrapedia* and *Coelioxoides*.

Michener and Lange (1958) published observations recorded by Alvaro Wille and Howell V. Daly on the nesting biology of *Tetrapedia maura* Cresson in Costa Rica, making possible behavioral comparisons of the two species. Camillo (2000) presented biological information of *Tetrapedia curvitarsis* Friese obtained from trap nests in southeastern Brazil.

The names of the authors to this article appear alphabetically. Each of us has pursued the investigations independently part of the time. IAS carried out observations at the Bee Laboratory at the University of São Paulo in São Paulo, Brazil, from November 1998 to December 2000. GARMś biological studies were at Ribeirão Preto from September 1998 to March 1999. JGR investigated the immature stages of host and parasite sent by the

other authors at his laboratory at the American Museum of Natural History in New York. IAS and GARM were primarily responsible for the biology sections of this paper, and JGRś main contributions were the sections on oocytes and immatures. After each author drafted sections of the manuscript, we gathered at the University of São Paulo, January 15–21, 2001, to observe jointly the nesting of these bees, to resolve differences in interpretations of observations, and to synthesize the information that is presented in the completed article.

METHODS AND TERMINOLOGY

Biological observations on *Tetrapedia diversipes* and *Coelioxoides waltheriae* were conducted at two sites. IAS saw females of *Tetrapedia diversipes* nesting in abandoned beetle holes in dead tree trunks first in December 1997 at the Bee Laboratory on the campus of the University of São Paulo (23°33′S, 46°43′W) in the city of São Paulo. Other species of solitary bees and wasps were also nesting in these trunks. In November 1998, she provided trap nests in a covered area to maintain and extend the nesting site possibilities of the bees and to allow the study of their biology and development. The trap nests consisted of circular holes, 3–10 mm in diameter, drilled 5–12 cm deep into pieces of wood. She lined each bore, oriented approximately horizontally, with a small piece of rolled paper so that she could later extricate the roll (now a tube) periodically with forceps and unroll it to reveal the bee nest. When a nest was permanently removed, a new piece of rolled paper was inserted in the hole. Extricated nests were placed in individual Petri dishes and kept in the laboratory, so that the emergence and sexes of adults could be registered. The bees were then either released near the nest area or preserved for the reference collection. IAS spent about 70 hours during spring and summer 1998/1999 and 1999/2000 observing females constructing and provisioning their nests. Recorded were the time that the female spends outside collecting provisions and the various construction materials (sand, oil) and the time that the female spends inside the nest to unload and manipulate each material. Nine

samples of pollen from the *Tetrapedia* nests were taken between December 1998 and January 1999 in São Paulo; they were acetolized and then analyzed. To mark specimens, she first refrigerated the bee and then dabbed its mesoscutum with colored paint.

More complete data on the sequence of nest construction and provisioning were gathered at this site during January 2001, when the three authors spent about 30 hours watching female activities of both hosts and parasites. With help of an ophthalmoscope we were able to clarify the movements inside the nests and acquire more data about cell construction and provisioning rhythms. Examination of opened nests enabled us to piece together how host females construct and provision cells and to make observations on the behavior of immature stages.

At the second site, in Ribeirão Preto, São Paulo, Brazil, GARM made observations in the backyard of a house in a suburban area at the western edge of town (21°10′S, 47°52′W). The plants in this area were mostly ornamentals, with weeds growing in a few vacant lots. He found females of *Tetrapedia diversipes* nesting in abandoned beetle holes in a standing stump (about 1.3 m tall) of a dead orange tree. To obtain data on the nesting biology of *Tetrapedia*, he bundled together seven trap nests and attached them horizontally to the stump on November 28, 1998. Each trap nest consisted of two rectangular pieces of wood firmly taped together, in which a round hole had been drilled through the contacting surfaces of the two pieces. The holes measured 4 mm in diameter and 57 mm long. Two days after the trap nests were deployed, *Tetrapedia* females had already occupied four of them, and one nest was being provisioned. Between November 30 and December 9, GARM watched the trap nests with the help of an ophthalmoscope. On December 9, he removed for dissection three nests, each containing a fully provisioned cell, and added two empty trap nests to the bundle. When checked again on February 8, 1999, the trap nests contained four provisioned nests, which were taken out and dissected. He obtained a total of 10 brood cells in seven trap nests at this locality.

Adult voucher specimens and vacated nests are preserved dry in the bee collection

of the Bee Laboratory at the University of São Paulo and in the Department of Zoology of the Universidade Federal do Paraná. Immature stages, preserved in Kahleś solution, are in the custody of IAS, Bee Laboratory, Universidade de São Paulo.

The scanning electron microscope (SEM) images of the female hind legs were made at the University of São Paulo. The legs of dry specimens were cut and placed directly on the stubs with double-face adhesive tape. They were then coated with gold palladium and examined with the SEM. For study of the immature stages at the American Museum of Natural History, the first instar and egg of *Coelioxoides waltheriae* and the egg of *Tetrapedia diversipes* were critical-point dried, coated with gold palladium, and then examined with an SEM.

For the preliminary chemical analysis of nest oils, a nest tube was removed from a trap nest, wrapped in cellophane, and transported to the Chemistry Institute of the Campinas State University in an icebox.

In the description of the pupa of *Tetrapedia diversipes*, the term careotubercle is introduced. It is derived from *careo* or *caritus*, Latin for "to feel the want of . . . " referring to pupal tubercles that do not contain developing adult structures, such as setae, but presumably have some other, yet unknown, function(s). Such tubercles appear empty on pupae in which the adult has partly developed. All other leg tubercles in this species accommodate developing adult setae. The paired mesoscutal tubercles of the Melectini are also careotubercles (Rozen, 2000).

In the descriptions of pupae, T stands for metasomal tergum, S for metasomal sternum, with following Arabic numerals indicating metasomal segments (e.g., T3 indicates third metasomal tergum; S6, sixth metasomal sternum). In locality citations, IBUSP stands for Instituto de Biociências da Universidade de São Paulo. Time is recorded as Standard Time, not Daylight Saving Time.

BIOLOGY OF *TETRAPEDIA DIVERSIPES*

ADULT ACTIVITY

In the city of São Paulo, adult females of *Tetrapedia diversipes* are active from the middle of October through May (no infor-mation available for males), while in Ribeirão Preto they start nesting slightly earlier (September). During the colder and drier months (June through August), the bees remain inactive as mature larvae in diapause, at least in most cases.

Females start flying about 7 A.M. and are usually fully active in the nesting area by 8 a.m. They cease activity around 6–7 P.M. During rainy or cloudy weather, females remain inside their nests. Foraging trips are short or long excursions, with females returning with pollen, oil, or nesting material on their scopal hairs (figs. 1–4), which are restricted to the tibial apex and basitarsus of the hind legs. Their flight in the vicinity of the nesting site is relatively slow, with hind legs hanging low.
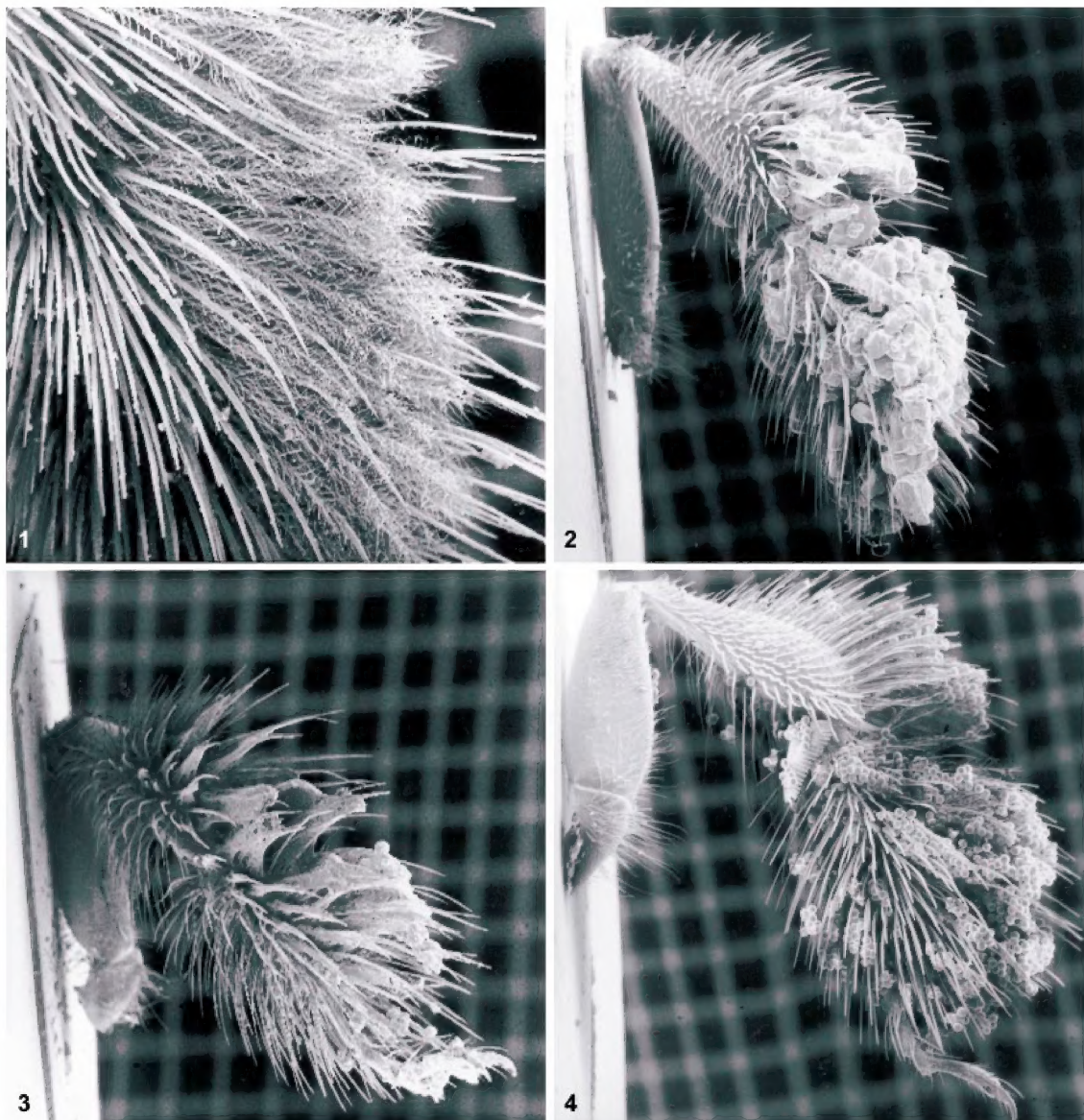
We saw no mating at the nest sites, nor did we observe males searching for females there. As usual for many bee groups, mating might be occurring on the flowers. On the other hand, considering that males are known to collect floral oils (see Pollen and Oil Sources below), males, individually or even in leks, might be able to attract females for mating.

Females of *Tetrapedia diversipes* sleep inside their nests or, in the case of non-nesting females, in empty tunnels. They apparently rest at an intermediate position along the length of the tunnel with their heads directed toward the rear.

From November 1999 to February 2000, IAS observed that males aggregated daily in mid-afternoon to spend the night on the same dried stems of a shrub within 5 m of the nests. They held the stems only with their mandibles and left their bodies and legs suspended (fig. 5), not, or only casually, in contact with the stems.

NESTING BIOLOGY

Tetrapedia diversipes is a solitary bee, with only one female to a nest. Because most nests consist of few cells, females obviously construct more then one nest during their lives. For example, in burrows that were 5 cm long (N = 17), females constructed 2 to 3 cells, closed the nests, and began searching for new nest places. Although no quantitative data on burrow di-

Figs. 1–4. Female hind legs of *Tetrapedia diversipes*. **1.** Close-up of scopal hairs, without nest material or provisions, showing long, coarse setae and short, multibranched, fine setae that serve as oil mops when carrying oils. **2.** Leg with scopa carrying sand grains for nest construction. **3.** Leg with scopa carrying oil. **4.** Leg with scopa carrying pollen.

ameters were gathered, *Tetrapedia* used burrows varying from 3–10 mm in diameter in São Paul and seemed to prefer those that were 4–6 mm in diameter.

Despite the fact that several of the wood blocks containing nests had many holes close to each other, females, both marked and unmarked, recognized their own nests immediately upon return. We observed little interaction among the many females that were nesting close to one another. However, females searching for nesting places sometimes entered active nests and were invariably expelled by the occupants. If the occupant was within the nest, she attacked the intruder with open mandibles; if the occupant returned to

Fig. 5.   Males aggregated where they came every afternoon to sleep on shrub stems close to the nesting area in the summer of 1999.

find the intruder within, she dragged the intruder to the entrance.

In *Tetrapedia*, the reuse of the old nest holes by subsequent generations is relatively common. The new female occupant cleans the hole by removing the old feces and most of the sandy material, which are easily detected on the ground in front of the nest entrance; she then begins nest construction itself. Sometimes females use holes that had been previously occupied by other species. Once in São Paulo, we noticed remnants of cells of *Anthodioctes megachiloides* Holmberg (Megachilidae) associated with a *Tetrapedia* nest, and twice we found *Trypoxylon* (Crabronidae) cocoons behind *Tetrapedia*
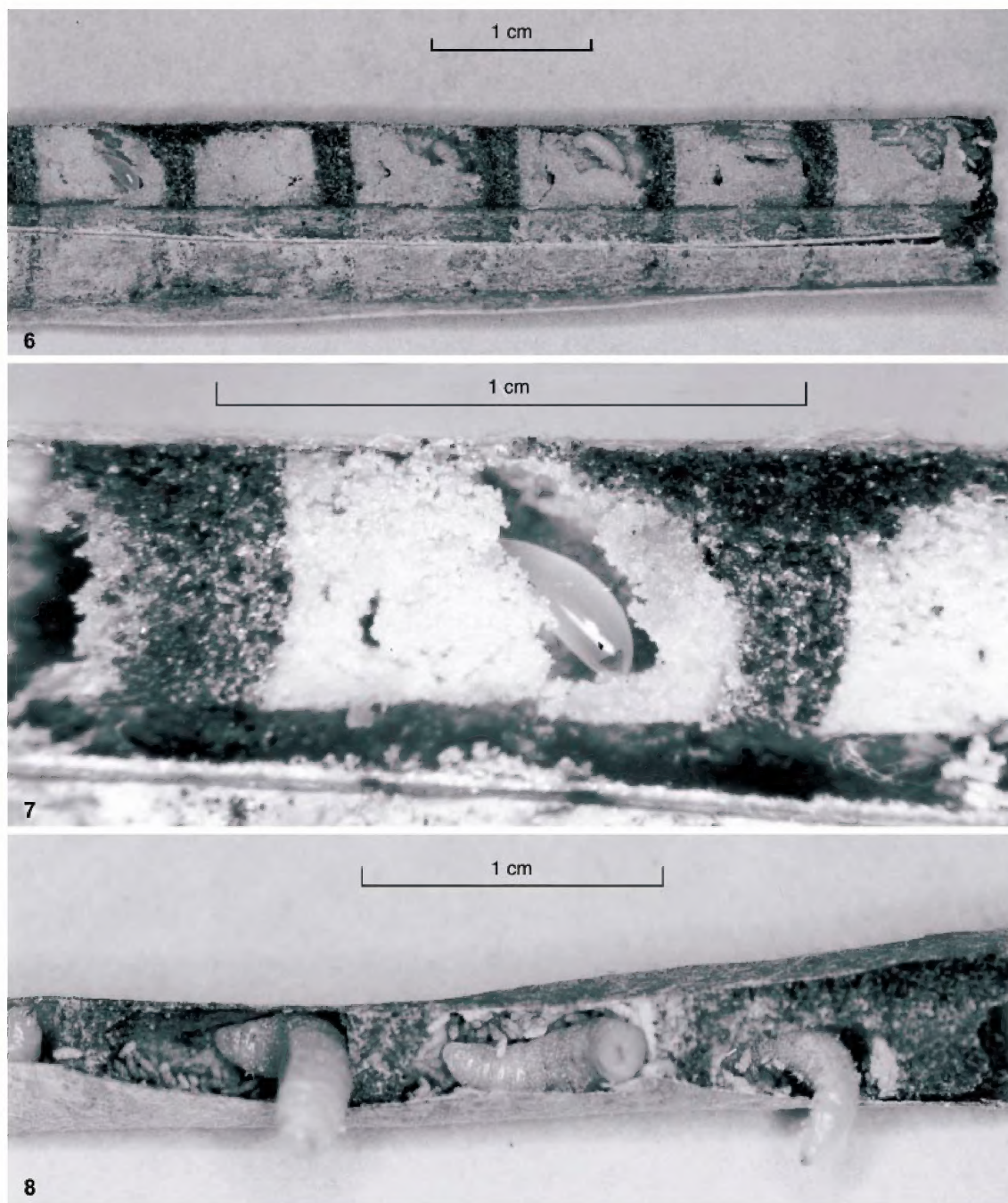
cells. Occasionally we discovered a nest that had been abandoned before completion (perhaps the female had died while foraging), and another *Tetrapedia* female occupied the hole some days later. Such composite nests sometimes had immatures in two developmental sequences.

NEST STRUCTURE

As in other trap-nesting bees and wasps, the nests built by *Tetrapedia diversipes* consist of a variable number of cells (from one to seven per nest; five to seven cells commonly in burrows 10–12 cm long) arranged linearly and usually occupying the full diameter of the hole (figs. 6–8). Most nests seem to consist of the cell series and an entrance passageway that is closed on nest completion with an exterior, outwardly concave septum, the nest closure made from soil and oil. Of six nests sampled at random, the lengths of the passageway behind the nest entrances were 6, 7, 8, 10, 12, and 15 mm, respectively. A seventh nest appeared to have a passageway of only 1 mm. However, in this case as well as in a number of other nests, we are uncertain as to whether a nest closure may be absent altogether or to whether a distinction can be made between the nest closure and the closure to the outermost cell in a series (see below). The entrance passageway behind the nest closure is usually partly filled with light reddish soil particles (mostly sand grains) that are loose and easily separated with forceps, in contrast to the cemented closure materials. In long passageways this loose material is mostly attached to the nest closure, with the area behind it open. We have not observed intercalary spaces between cells.

In most nests that occupied the whole length of the burrow (i.e., nests that would not have space for an additional outer cell), the outer closure was a little recess from the rim of the entrance, perhaps by 3–7 mm.

Each cell is delimited at both ends by a septum (or partition) made with a mixture of sand and oil collected from flowers. The outer surface of the septum is strongly concave, like that of the nest closure, while the inner surface of the septum sealing the cell is more or less flat, perpendicular to the long axis of

Figs. 6–8.   Nests of *Tetrapedia diversipes*. **6.** Paper roll from nest, cut open to reveal six cells, front end of nest to the left. Notice individuals become older to the right. **7.** Close-up of first cell containing egg toward rear of cell. **8.** Nest containing nearly mature larvae, front of nest to the right. Fecal pellets clearly visible in two of the cells.

the cell. We could not detect a spiral configuration to the inner surface of the septum, as is characteristic of cell closures of many bee taxa. The outer surface is somewhat shinier than the inner one and seems to provide much of the strength of the mixture after it hardens. When JGR attempted to penetrate the outer surface with sharp forceps, the tips met considerable resistance; the sand grains were bound to one another. This raised the question of how emerging adults could penetrate cell and nest closures with their mandibles to exit nests. However, when he attempted to use forceps on the inner surface, he found that the sand grains were not so strongly bound and could be more easily removed. We presume that the female applies oil, some secretion, or a combination of the two to the closure after it is in place, and that this material serves to glue the outer part of the closure, making it less accessible to parasites and predators. Although we have not observed adults exiting from their natal cells, remains of vacated cells suggest that adults use their mandibles to create a hole in the thinnest (central) part of the closure, leaving only the rim in place.

The configuration of the septa obviously determines the shape of the cell. Hence, the front end of the cell is flat and its rear concave. The walls of the cells are lined with a thin, transparent layer of floral oils. In nests built in wider holes, the bees line the cell walls with a fine layer of the same material as is used for the septa. Cell lengths varied from 10 to 15 mm and were inversely correlated with the diameter of the tunnel.

Cell partitions, including the septum at the rear end of the nest, consist of a single thin layer, 0.5–1.5 mm thick. The septum sealing the outermost cell tends to be thicker than the other partitions and is usually composed of three layers, two hard, compact layers and a middle layer of loose sand grains (in one case, the outer septum had five layers, i.e., three compact layers separated by two layers of loose sand). The sand grains in these intermediate layers are not completely loose, but have a thin film of oil on them. The thickness of the outer septum varies from 1 to 4 mm at its middle portion. After it hardens, the mixture used as nesting material is

waterproof, and a cell closure immersed for more than 24 hours in water did not dissolve.

The sand that the females transport into the nests for cell partitions and nest closures was analyzed for two nests, one from São Paulo and the other from the Ribeirão Preto. Both were composed of 100% sand. That from the São Paulo campus consisted of medium (particle size <0.50 mm, >0.25 mm) to nearly very fine sand (particle size >0.053 mm), and that from Ribeirão Preto ranged from coarse (particle size >0.50 mm) to fine sand (particle size >0.106 mm). Since most soils are not this coarse textured, we think that the females must search for special areas where coarse-textured soils are to be found, that is, areas where finer soil is washed or blown away. We saw no females landing on the ground near the nesting areas. Casual observations of soil from other nests suggest materials finer than sand may also be incorporated.

PROVISIONS AND EGG PLACEMENT

Fully provisioned cells of *Tetrapedia* are unusual in that the food mass is not formed into a loaf or sphere, as is the case with most bees that have nonliquid provisions. Rather, the pollen mass occupies the entire chamber (figs. 6, 7), the largest and densest part being against the front closure. Behind this mass are found one to three hollow cavities within the remaining provisions. Females always add a variable amount of floral oil to the pollen, so that the provisions appear darker and wetter where the oil is more abundant. Within a given cell, one finds portions of the provisions containing a larger amount of oil interspersed among sections with little or no oil. Sometimes the portions with oil form distinct, vertical bands in the pollen mass. We have no evidence that females add nectar to the pollen.

The egg is always found within one of the cavities within the pollen mass. In pollen masses with more than one cavity, we never observed an egg in the forwardmost cavity. The egg seems to have a stereotypic orientation within the cavity, with its posterior end down and with the more or less vertical egg leaning forward against the provisions, but these matters deserve further study.

Sequence of Cell Construction,
Provisioning, and Oviposition

After choosing a hole suitable for nesting, the female starts bringing in nesting material (soil particles, mostly fine sand grains, mixed with floral oils) to build the rear septum. Although not seen during this study, GARM and JGR have repeatedly observed females of several species of *Tetrapedia* landing on the ground surface, especially on dry spots in which sand grains seem to have accumulated, to collect soil. Using their mid- and hind legs, they shuffle the soil and apparently mix the particles with the oil on their scopae.

Oil foraging probably precedes soil collecting; however, we did not study the bee behavior while collecting oils on flowers. Judging from the short duration of some of the soil-collecting trips, it seems possible that oil gathered in a single trip could be used in more than one soil-collecting trip. This is also suggested by those loads containing a large proportion of soil particles with very little oil associated with them.

Females bring in nesting materials to the nest on their scopae (figs. 2–4). A female loaded with nesting material can be easily recognized by the dark brown color of her legs, in contrast to the yellow or orange color in females carrying pollen or to the shiny aspect of legs loaded mostly with oil.

The female enters her nest headfirst, walks to its rear part, and antennates the area under construction. On a number of occasions, GARM observed females licking the surface of the partition under construction before laying down a new layer of material, as if applying a secretion with the proboscis. After this, the female turns around and unloads the material. In narrow nests (under 4.5 mm in diameter), the females are unable to make turns within the tunnel; they have to come out and then reenter walking backwards.

After unloading and before departing on a new trip, the female spends a few minutes packing (i.e., manipulating and consolidating) the material with the tip of the metasoma, probably using the pygidial plate and the fimbria on the apex of the 5th tergum. Despite having a reduced pygidial plate, a feature in common with other bees that do not excavate their nests in the ground, the basal portion of the plate is well developed in *Tetrapedia*. Also, the fimbria on T5 contains several thick, brushlike bristles along the apical margin of the tergum, which are probably directly involved in packing. Lack of these specialized bristles in old, worn-out females suggests that they are heavily used.

On completion of the rear septum, the next step consists of applying one to two layers of pure oil (i.e., oil not mixed with sand) over the inner cell walls. In recently sealed cells, this oily layer possesses a waxy consistency when scraped off, but it eventually hardens and becomes varnishlike in appearance.

Although we did not gather quantitative data on the time of day when new nests are started and the amount of time spent preparing a cell for provisioning, it seems that most females start looking for a suitable burrow in late morning or early afternoon. Cells initiated around this time are usually ready for provisioning at the end of the afternoon. In this case, however, females will start bringing in pollen only the following morning.

To collect nesting materials in general, females spent roughly 20 min outside the nest per trip (based on data gathered by IAS), although exact times varied widely: *pollen:* mean = 21, range 2–57 min (N = 58); *oil:* mean = 21, range 4–66 min (N = 17); *sand:* mean = 17.5, range 2–51 min (N = 29). To unload the materials inside the nest, females spent on average 5 min for the pollen (range 1–52 min; N = 78), 19 min for oil (range 3–44 min; N = 19), and 11 min for sand (range 1–29 min; N = 29). In 70% of the observations, returning females loaded with pollen spent not more then 3 min inside the nest and left for the next trip with clean scopae. We suspect the disparity in time between unloading pollen compared with unloading oil and sand results from the fact that pollen is simply dumped in the open cell by the females returning from most pollen-foraging trips. Cell provisioning is unusually slow in *Tetrapedia diversipes*. Based on a sample of six cells, followed from start of provisioning to oviposition in January 2001, each cell takes approximately 5 days to be fully provisioned (no variation was detected among the six females).

In a given day, pollen foraging is concen-

trated in the morning. Eighty-six percent of the 108 pollen trips recorded during January 16–20, 2001, occurred before noon. The behavioral sequence involved in pollen unloading and manipulating is similar to that described above for construction of septa, except that females were not observed licking the pollen mass when first entering the nest with a new pollen load.

During cell provisioning, floral oils are also added to the pollen mass. Every day one or two oil loads are brought in and mixed with the outer layer of the pollen mass. Foraging for oil to be used as food occurs in middle to late afternoon and usually corresponds to the last foraging trips made by a provisioning female in a given day.

Despite the fact that in *Tetrapedia* the egg is laid within the food mass and usually at the rear of the cell, egg laying takes place only after the cell has been fully supplied with pollen. A fully provisioned cell before egg deposition contains a compact food mass occupying approximately the inner half to two-thirds of the cell length.

Data gathered from a nest opened shortly after the female had laid an egg and while she still was within the food mass gave us an opportunity to understand the final steps in cell construction. In this nest, the egg was in a partly open chamber at the back of the cell. It was standing on its posterior end and had the anterior end inclined toward the entrance. The female was in a larger chamber in front of the egg chamber, with her head toward the cell entrance and with a large supply of pollen loosely spread over the bottom and lateral cell walls in front of her. Between the two chambers, the pollen mass formed a small mound, indicating that the female was in the process of building with her metasomal apex a pollen partition closing off the egg chamber. We deduce that after completing this partition, she crawls over the loose pollen mass and then starts packing it with her metasoma. At the end, the outer surface of the pollen mass will be shaped as a smooth vertical wall on which the female applies the soil–oil mixture to form the cell closure. By this process, an additional chamber is formed within the food mass in the space previously occupied by the female's metasoma when she was building the partition in front of the egg chamber.

Apparently, the construction of a final cell closure starts soon after the female finishes packing the food mass. The soil–oil mixture is applied directly over the pollen mass, starting around the edges of the cell circumference and then on the center portion. We did not gather data on the number of loads used for each cell partition, but a few observations suggest averages of four to eight loads.

The soil–oil mixture is friable and soft when first laid down, but becomes hard and brittle in a few hours. Some substance added when females apply their proboscis to the septum under construction might accelerate hardening of the soil–oil mixture. This is a matter of considerable importance with respect to egg deposition by *Coelioxoides waltheriae*, as discussed in the biology of that species, below.

POLLEN AND OIL SOURCES

Our field study did not include an investigation into the sources of the larval provisions. However, from the literature, *Tetrapedia* is known to collect pollen from the flowers of *Ludwigia* (Onagraceae), Asteraceae, and Cactaceae (Silveira et al., 1993; Schlindwein, 1998; Alves-dos-Santos, 1999). Analyses of nine pollen samples from nests that IAS collected from the University of São Paulo campus in the summer 1998/1999 showed that the bees preferred pollen of Euphorbiaceae. In six samples we found pure pollen of *Croton* (Euphorbiaceae), and in three samples this pollen was mixed with that of Asteraceae. We are uncertain of the significance of these samples, since the bees may be opportunists, collecting pollen from sources in an urban area where there are many ornamental plants.

Vogel (1974) first suggested that *Tetrapedia* collects floral oil just on the basis of the modification on the anterior basitarsus, the comb. Later, Neff and Simpson (1981) confirmed this hypothesis, attesting that *Tetrapedia* collects oil from the flowers of Malpighiaceae. Their observation was substantiated by many authors, for example, Buchmann (1987), Rego and Albuquerque (1989), Simpson et al. (1990), and Pedro (1994) (this

last work was conducted in a cerrado area close to Ribeirão Preto). Neff and Simpson (1981) have also called attention to the finely branched hairs present along the posterior portions of the hind barsitarsi and apices of the hind tibiae of both female and male *Tetrapedia* (see fig. 1). They suggested that presence of these specialized hairs constituted further evidence that *Tetrapedia* was an oil-collector. Preliminary chemical analyses of one nest of *Tetrapedia diversipes* from the campus of the University of São Paulo showed the presence of oil in the pollen mass and on cell walls. The composition of the free saturated fatty acids was more complex on the cell wall than in the pollen mass, suggesting the possibility that this might relate to the observations (see previous section) of the female applying her proboscis to the cell partitions while constructing them. The oil found in the nest analyzed had no diglycerides, substances abundant in other floral oils, as found in *Calceolaria pavonii* Benth. (Scrophulariaceae) by Vogel (1974), *Byrsonima crassifolia* (L.) Kunth (Malpighiaceae) by Vinson et al. (1997) and, *Oncidium pubes* Lindl. (Orchidaceae) by Reis et al. (2000). Our preliminary chemical analysis looked for but did not find β-acetoxy fatty acids.

The plant (or plants) from which *Tetrapedia diversipes* gathers oil is not certainly known. Some evidence suggests that this reward may not come from the flowers of Malpighiaceae, as predicted earlier by the authors cited above. We saw two females returning to the nest, each with a pollinarium on her clypeus. One of these specimens was preserved and the pollinarium later identified by Rodrigo Singer as from the subtribe Oncidiinae (Orchidaceae). Schlindwein (1998) found pollinaria of an *Oncidium* sp. on the head of *Tetrapedia rugulosa* Friese and thought that the bee was using this orchid for oil. Alves-dos-Santos (1999) discovered *T. rugulosa* collecting oil from *Sysirinchium* sp. (Iridaceae). Also, Vogel and Machado (1991) reported oil-collecting on flowers of *Angelonia* (Scrophulariaceae) by females and males (!) of a *Tetrapedia* species (tentatively identified as *T. rugulosa*). These last authors called attention to the unusual behavior of the males, which carried the oil on the finely branched hairs of their hind tibiae and basi-

tarsi.[4] Similar observations of a male of *Tetrapedia diversipes* collecting oil on flowers of *Oncidium paranaense* Kraenzl. were recently reported by Singer and Cocucci (1999). (IAS was able to recover their male specimen from the collection at the University of São Paulo, confirmed its gender, and noted the oil on these hairs.)

Many plants on the University of São Paulo campus produce oil potentially available to *Tetrapedia*, such as Orchidaceae (*Oncidium*) and Malpighiaceae from the Botanical Garden and Iridaceae that are abundant over the whole campus. Future chemical analyses of these supposed oil-source flowers should be compared to the substance found in the *Tetrapedia* nests.

DEVELOPMENT

Based on observations of a few developing eggs and anatomical evidence cited below, we conclude that the first larval instar of *Tetrapedia diversipes* is pharate within the egg chorion. Transition from the embryonic stage to the first instar takes place at some time before the chorion ruptures on each side along the spiracular line. This partial rupture of the chorion presumably results from increase in body size because of ingestion of embryonic fluid. The chorion is finally shed with the first instar exuviae during molting to the second instar, all before any provisions are consumed.

Three flattened cast larval exuviae, found adhering to one another in a cell, provided anatomical evidence in line with these observations. The exuviae represented the first three instars. The cast skin of the first instar was unsclerotized except for the mandibular apices, which were identical in shape to those of the first instar (see fig. 32) removed from the chorion (see the section on Descriptions of Immature Stages). The head capsule was entirely flattened (due to lack of sclerotization) with features unrecognizable ex-

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[4] This was a remarkable observation, to our knowledge without counterpart among bee taxa with scopae. Michener's (2000: 675) statement that males of *Tetrapedia* presumably do not carry oil appears to be incorrect. We know of no other bee in which the males have modified hairs on the hind tibiae and basitarsi and use them to transport substances. These observations invite investigation.

cept for the mandibular apices; none of the head ridges was pigmented, and the labral sclerite was not evident. The two subsequent instars differed from the first in that the head capsules were sclerotized and pigmented, with the internal head ridges, mandibles, stipes, cardines, and labral sclerites darkly pigmented. The presence of these ridges permitted the lower part of both heads to hold their shape so that they were not flattened even though the dorsal parts of the crania had collapsed as the result of ecdysial splitting.

The weakly sclerotized, unpigmented first instar contrasting with the sclerotized, pigmented subsequent instars is taken as evidence that the first instar is nonfeeding and remains within the chorion. The existence of first instars being encased in the egg chorions for most, if not all, of the first stadia has been observed by JGR in a number of taxa (*Svastra*, Rozen, 1964; *Anthophora*, Rozen, 1969a; *Ptiloglossa*, Rozen, 1984a; *Centris*, Rozen and Buchmann, 1990) whose eggs are nonreflective due to chorionic microstructure. Hackwell and Stephen (1966) reported that *Nomia* had five larval instars, the first enclosed in the chorion. Torchio (1989a, 1989b) described in excellent detail the eclosion of *Osmia* and *Stelis*, both of which have pharate (or partially pharate) first instars. Similarly, Rust et al. (1989) reported that another species of *Osmia* had five larval instars with the first pharate and nonfeeding. More recently, Trostle and Torchio (1994) reported that two species of *Megachile* had five larval instars, and normally both species eclosed as second instars although one occasionally hatched as a first instar. The widespread appearance of pharate first instars in bees raises the question whether it might be characteristic of bees in general. It presumably is not characteristic of all bees, however, for it is undetected in the Nomadinae or in *Coelioxoides*. Although Rozen (1967) stated that *Dioxys* had four larval instars, Rozen and Favreau (1967: fig. 5) depicted ''an egg that was about to hatch'' that, in hindsight, could well have been a pharate first instar.
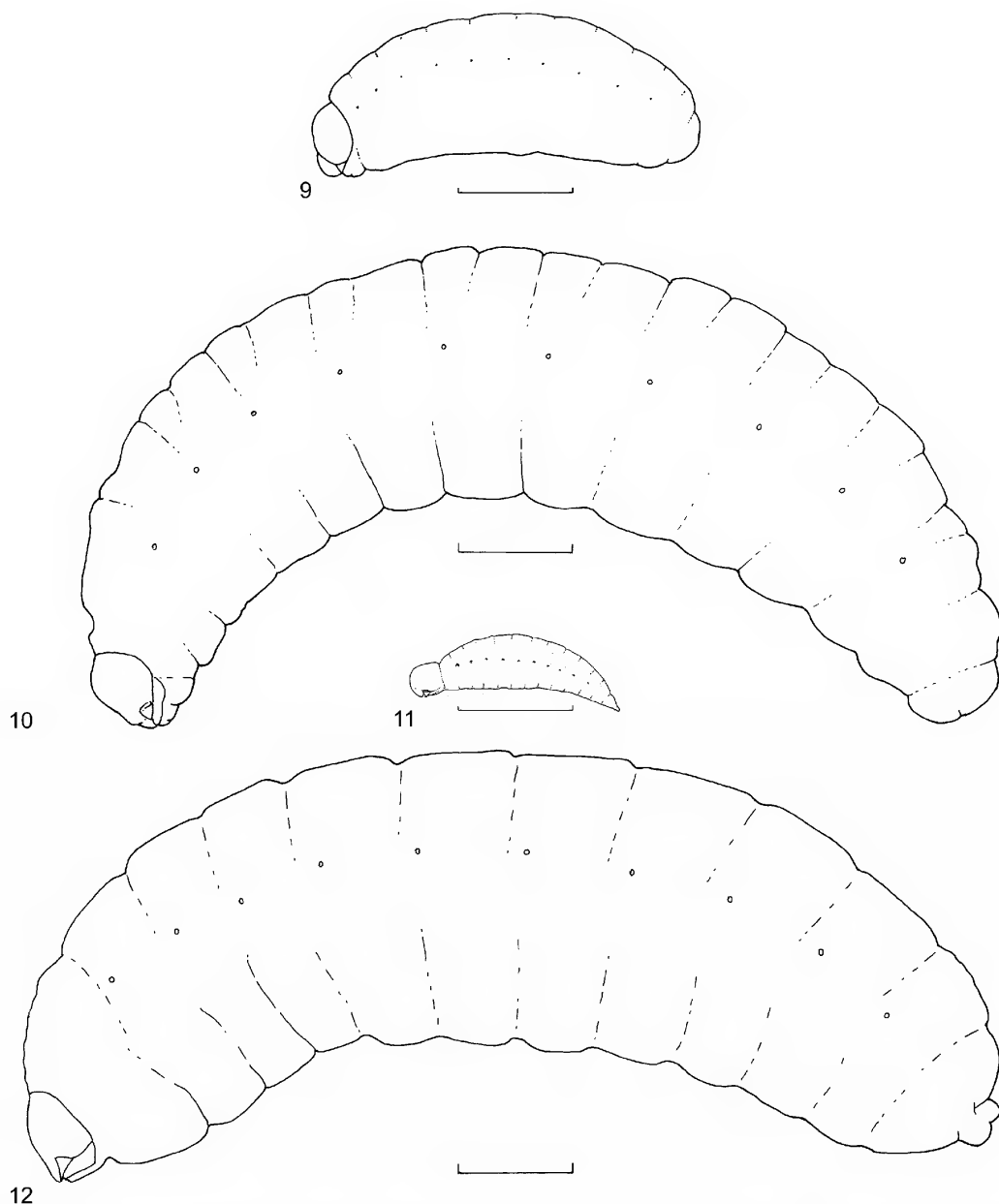
That the recovered three exuviae of *Tetrapedia diversipes* were superimposed with the first instar lowest indicated that the larva does not move in relation to its food while it feeds, at least for the first three instars. Such sessile early instars are encountered in many groups, including most Panurginae, Halictinae, and Megachilidae. However, such behavior is not characteristic of many groups of Apidae such as the Emphorini, Exomalopsini, Eucerini, and Centridini.

The anatomical differences between the second to fifth instars of *Tetrapedia diversipes* apparently are slight judging by these cast exuviae, a single fourth instar, and numerous fifth instars. Surprisingly, relatively little change in size of the mandibles occurs from one instar to the next and reflects the fact that head size changes little from the first to final larval instar (figs. 9, 10). This in turn probably relates to the fact that the first instar is unusually large, having hatched from a giant egg (see the section Discussion of Eggs of *Tetrapedia* and *Coelioxoides*). Changes in mandibular shape are depicted in figures 13–19, all of which are shown at the same magnification.

The difference in the mandibular apices exhibited by an early fifth instar (fig. 17) and a mature fifth instar (figs. 18, 19) is noted. The much shorter, rounded teeth of the latter presumably result from abrasion. The few cell partitions examined by JGR bore no mandibular scrape marks, but GARM has noted a high number of marks on the lining of cell walls in nests from Ribeirão Preto. Rozen (1994b) observed apical mandibular wear in last instars of *Ancylandrena*. Norden et al. (1980) reported that mature *Anthophora* larvae normally ate the cell lining composed of Dufour gland secretions after consuming the provisions. Rozen (1970, 1973, 1977) and McGinley and Rozen (1987) concluded that larvae of the Fideliinae ingest sand (in the case of *Pararhophites*, sand presumably mixed with nectar) after consuming their provisions and then defecate sand on the insides of their cocoons, presumably to strengthen them. Thus, fully fed mature bee larvae have varied behaviors, some of which may contribute to mandibular wear as exhibited in *Tetrapedia*.

As indicated above, the fact that young larvae are found with the earlier exuviae attached posteroventrally on their abdomens suggests that young larvae do not crawl. Last instars obviously do move about the cell to reach provisions. Defecation begins when the

Figs. 9–12. Comparisons of body sizes of first and last larval instars of *Tetrapedia diversipes* (**9, 10**) and *Coelioxoides waltheriae* (**11, 12**), all drawn to same scale, lateral view. Scale line = 1.0 mm.

larva reaches the last instar but while it is still feeding. GARM observed the onset of defecation to be a few hours after the molt to the last larval instar. The feces, spindle-shaped pellets, appear to be scattered throughout the cavity but may be pushed to the cell rear before pupation. The last feces to be discharged (after all provisions are con-

sumed) are composed of a black, tarry material. *Tetrapedia* larvae do not spin cocoons.

Unlike those of many bees, the pupae of *Tetrapedia diversipes* seem to be randomly oriented with respect to which direction they face. A sample of 13 pupae revealed that 8 faced the rear of their cells, while the remaining 5 faced their cell closures.

Figs. 13–19. Right mandible of larval instars of *Tetrapedia diversipes*, outer view, drawn to same scale: first (**13**), second (**14**), third (**15**), fourth (**16**), fifth, before maturity (**17**), and two views of fifth (postdefecating), showing variation (**18, 19**).

Figs. 20–22. Right mandible of larval instars of *Coelioxoides waltheriae*, outer view, drawn to same scale as figs. 13–22: first (**20**), second (**21**), and fifth (postdefecating) (**22**).

IAS kept 10 nests (containing 39 brood cells) in the laboratory from which 31 bees emerged; 16 were females and 15 males. Although the total sex ratio was nearly equal, it varied from nest to nest. For example, from one nest, 7 males and no females emerged, while the total emergence from another nest consisted of 3 females. The emergence of the adults from a nest can take 2–4 days or can be brief, within an interval of just few minutes. For example, the 7 males mentioned above emerged from 4:40 to 4:50 P.M. on February 26, 1999; 5 individuals from another nest took 4 days (February 28, 1999 to March 3, 1999) to emerge.

The mortality rate was relatively low in the first year in São Paulo. Less then 20% of brood cells from extricated nests showed mortality of immatures (young larva, mature larva, or pupae). Although mortality rate was not measured in the second year, it was visibly higher, due mainly to the increase in mite infestations and the presence of *Melittobia* inside the cells (this wasp not present earlier). Fungi also attacked the provisions during the second year.

The developmental rates of the early instars of *Tetrapedia diversipes* are discussed in comparison with those of *Coelioxoides waltheriae* as a separate section after Development of the latter species.

COMPARISON WITH OTHER SPECIES OF *TETRAPEDIA*

Michener and Lange (1958) presented information on the foraging behavior and nesting biology of *Tetrapedia maura*. They presented notes recorded by Alvaro Wille and Howell V. Daly, who made the original observations in Costa Rica. The nesting biologies of the two species agree in most respects. However, the preformed cavities used by *T. maura* were probably beetle burrows, most likely those of Buprestidae judging from their oval shape in cross section. In addition, the sections of the burrows containing cells were vertical, in contrast to the essentially horizontal cells of *T. diversipes* in trap nests. The eggs were deposited vertically in the cells, which suggests that in both species eggs may be oriented in relation to gravity rather than to the long axis of the cell.

They reported that the burrows were narrowed by ''hard gray material'' which formed the cells in one nest. We think that this was probably soil that the female had imported and may have been in greater evidence along the sides of the tunnel than in the nests of *T. diversipes* because the burrow was oval and not circular in cross section, as were the artificial burrows of our trap nests. They also reported that a nest entrance was funnel-shaped and narrowed due to the placement of this material, a statement that suggests that the bees were reusing old nests. Considering our observations on *T. diversipes*, what Michener and Lange interpreted as

a narrow nest entrance was most likely the hole made on the outer partition by a bee that emerged from the nest. Although there were several references to the bees "excavating" the nests and backing out of the tunnel with "large loads of wood powder", we suspect that this might have been remains of old cells or frass left behind by the beetle larva rather than debris from the bee excavating into solid wood. Because we know that *Coelioxoides* is a cleptoparasite of *T. diversipes*, we wonder, as did Michener (2000: 675), if the reported sighting of *Coelioxys* investigating the nest of *T. maura* was a misidentification.

Recently, Camillo (2000) published a summary of his studies on the biology of *Tetrapedia curvitarsis* using trap-nests. His observations on its nest architecture are, in general, similar to what we have found for *T. diversipes*. Although apparently not as slow as those of *T. diversipes*, females of *T. curvitarsis* also take more than one day to build, provision, and seal one brood cell (an average of 38 hours for two cells). The data presented by Camillo strongly suggest that immatures of *T. curvitarsis* enter diapause, probably as prepupae, during the dry, cold season. Bees from nests built at the end of the warm season (April and May) took about 90–180 days to emerge compared to 40–55 days from nests built earlier in the season. Three significant differences between our observations and those of Camillo are noteworthy. He stated that the nest materials (oil and sand) are brought in separate loads and then mixed inside the nest. Although some loads of *T. diversipes* contained mostly sand grains with little oil among them, we have no data suggesting that the females mix the materials inside the nest. Loads containing only oils were either applied directly on the nest walls or on the outer partition. Only when used as food was the oil mixed with pollen inside the nest. Camillo also stated that in *T. curvitarsis* the egg is laid soon after provisioning commenced. In this species, as in *T. diversipes*, the egg is usually placed in a chamber at the rear of the cell. The author might have taken this as evidence that the egg is laid before the cell has been fully provisioned. In our study, we opened several cells that were being provisioned, some of them already for more than 3 days, and none

contained an egg. Camillo also mentioned that in some nests the female starts constructing the cell closure before the cell has been fully provisioned. Similar to what Michener and Lange reported for *T. maura* (see above), Camillo's description of the partly built partition suggests that he most likely had seen old partitions that had been perforated by an emerging bee, therefore implying nest reuse, rather than fresh partition construction.

COMPARISON WITH OTHER NONCORBICULATE APINE BEES

Besides *Tetrapedia*, the only noncorbiculate apine bees known to nest in narrow pre-existing cavities (such as old beetle burrows or trap-nest bores) are *Ctenoplectra* (e.g., Rozen, 1978) and the subgenera *Xanthemisia* (Vinson et al., 1993; F.A. Silveira and G.A.R. Melo, unpubl.) and *Heterocentris* (including *Hemisiella*) (e.g., Jesus and Garófalo, 2000; Pereira et al., 1999) in *Centris*. These represent at least three (and possibly four) independent origins of narrow-cavity nesting, since *Xanthemisia* and *Heterocentris* are not closely related within *Centris* (Ayala, 1998). Although *Ctenoplectra* is quite distinct from *Tetrapedia*, a sister group relationship between the two cannot be totally discarded at this point (see below).

We considered whether nests made of imported soil and floral oils may preadapt a bee lineage to use narrow cavities for nesting because these four groups are the only cavity-nesting, noncorbiculate apines that use these nesting materials. (The behavior of *Xanthemisia* is somewhat distinct in that they mix the oils with fine wood particles scraped from the walls of the preexisting tunnels in which they nest.) Floral oils alone may be insufficient for sparking a transition from ground to cavity nesting since no Tapinotaspidini uses preexisting cavities; most collect oils (Michener, 2000) but do not plaster in cell walls that harden (Rozen, 1984b). However, it is unknown whether these oils are used in cell construction in this tribe. By similar reasoning, hardened thick cell walls alone do not seem to offer the evolutionary impetus to switch to cavity nesting since there are no cavity-nesting Emphorini and Anthophorini, nests of which have conspicuous, thick, plas-

tered cell walls; such walls are constructed with the use of water and perhaps nectar in some cases (Michener, 2000; Rozen, unpubl. notes). We suggest that studies of humidity conditions in cells that are lined either with floral oils or with a mixture of oil and soil might provide insight into this matter.

In Roig-Alsina and Michener's (1993) study, the Tetrapediini came out as a somewhat isolated group within the Apinae, showing no clear affinities with any other of the smaller groups. The somewhat similar nests built by *Tetrapedia* and *Ctenoplectra*, as well as the few, but relatively weak, similarities between their mature larvae (see Discussion of Mature Larvae of *Tetrapedia* and *Coelioxoides* below) could be seen as an indication of a close phylogenetic relationship between them. However, crucial information on nesting biology of *Ctenoplectra* is still lacking and until further data are gathered, a detailed comparison between that group and *Tetrapedia* is not possible.

BIOLOGY OF *COELIOXOIDES WALTHERIAE*

NEST SEARCHING AND OVIPOSITION

The annual period of adult activity of *Coelioxoides waltheriae* coincides with that of the host (November through May). We observed females of *C. waltheriae* flying slowly around the nest entrances from 8 A.M. to 6 P.M. They are attracted to the holes in the trap nests and apparently are able to recognize in flight nests of *Tetrapedia*. Although their flight may appear random, they monitor active nests. As a cell is nearing completion, they exhibit an increased interest in the nest, in that they more frequently inspect it. They may enter it but will depart immediately if the nest is unsuitable. On other occasions, if while hovering in front of an entrance, a parasite female detects a host female inside, the parasite may land close to the opening (about 10 cm away). She may stay there for a long period (sometimes more than 1 hour) facing the nest entrance, apparently waiting for an opportunity to enter the nest.

When a parasite female enters a nest and detects a host female inside, she immediately withdraws. We have never observed a cleptoparasite expel the nest owner. On a few oc-

casions, we have seen female *Tetrapedia* approach and apparently attack a *Coelioxoides* perched by a nearby nest.

Evidence suggests that *Coelioxoides waltheriae* oviposits only in cells that have been recently closed. We have never observed searching adults remaining beyond a second or two in nests with an open cell being provisioned. On two occasions when a parasite was showing great interest in a nest but was unable to enter it because of a guarding host female, we experimentally removed the hosts. Within a few minutes the parasites returned and entered those nests. They spent 6 and 10 minutes, respectively, before departing. When we inspected these cells, we found scars on the closure surface indicating that the parasite was unable to penetrate the closure material, which was hard.

On one occasion we were able to observe a successful oviposition by a *Coelioxoides* female. At 1:09 P.M. the nesting female brought in the first load of soil to construct the outer ring of the closure. Five and 14 min later, respectively, she returned with the second and third loads of soil. Approximately 4 min after her last return, a female *Coelioxoides* flew around the entrance and then landed about 10 cm from the nest, facing its entrance. Five minutes later, the parasite repositioned herself, again facing the entrance. Eleven minutes after that, the host female left, and almost immediately the female *Coelioxoides* entered the nest. When we looked inside, she had already reversed her position so that she was facing the entrance, with her venter up. Two minutes later, the host came back, entered the nest, and left in less than 30 seconds. We looked inside immediately to find that the parasite had not changed its position. She had made a large puncture through the cell closure apparently in the process of laying her egg. The size of hole was large enough to cause some pollen to spill forward. She soon started to use the tip of her metasoma to patch the hole and spent about 4 min doing this before finally departing. The female parasite had spent a total of 9 min within the nest. The host female, bringing in soil, returned nine minutes after the parasite's departure. She brought in an additional three loads of closing material.

From these observations and the fact that

host females tend to guard their cells after nest closure, we conclude that nests are vulnerable to parasite attack only before the closure material has hardened, at least for the closure of the outermost cell of a cell series. This conclusion is supported by the observation that the soft cell closure often bulges inwardly when penetrated by the parasite's ovipositor. These bulges harden and can often be observed in cells containing *Coelioxoides* mature larvae or pupae. Thus, the narrow window of opportunity for the parasite to oviposit would seem to be when the host is away gathering closure material.

Occasionally we found more than one *Coelioxoides* egg in a cell, and one cell contained four parasite eggs. We think these are cases of multiple parasitism where more than one parasite female introduced her egg into the cell.

DEVELOPMENT

In contrast to *Tetrapedia diversipes*, the first instar of *Coelioxoides waltheriae* is not pharate and inactive. We could detect no weakly sclerotized earlier larval exuviae in any of its cast chorions. The first instar described herein is truly the first and is active, observed attacking eggs and first instars of other *C. waltheriae* as well as host eggs. Further, our observations on two other first instar *Coelioxoides* less than two days after oviposition (as described and discussed in the following section) suggest there would scarcely be time within such a brief period for the development of both a first and second instar.

Although we have not observed eclosion in *Coelioxoides waltheriae*, cast chorions were intact except the anterior ends were torn, an indication that the larva emerges through this end.

The enormously broad labrum of the first instar, studded with sensilla, probably functions to detect the host egg and competitors; it may also may help form a seal permitting the first instar to suck the yolk from the host eggs. The second instar (based on a single cast exuviae) possesses sharp mandibles (fig. 21) that are not as elongate compared with basal width as those of the first (fig. 20), but which seem sufficiently sharp to be used in

attack. The anatomies of other instars have not been studied except for the last by which time the mandible has lost much of its curvature and has become irregularly serrate along both dorsal and ventral apical edges (fig. 22).

On January 18, 2001, we observed a female of *Coelioxoides waltheriae* enter a nest (coded Y1, fig. 24) of *Tetrapedia diversipes* at 1:42 P.M. and depart at 1:52 P.M. When dissected, the nest contained a *Coelioxoides* egg as well as the host eggs. When we observed the cell at 8:00 A.M. on January 20, an active first instar of the cleptoparasite was on top of the host eggs, a period of 40 hours between parasite oviposition and our observation of its first instar.

We were able to induce the larva to crawl by teasing it with a hair. It first contracts its posterior body segments and then, while expanding and slightly lifting them, it remains attached to the substrate at the extreme rear end. Consequently, the expanded part of the body sends the anterior part forward. Repetition of this behavior gives the impression of waves of body contractions from posterior to anterior. The rounded projection (fig. 34) at the abdominal apex apparently does not contract, but abdominal segments 9 and 10 are capable of considerable shortening. Thus, this projection may serve as a foot to stabilize the foreward thrust as the larva moves along. We observed, however, that the larva did not always push with this projection; the larval integument is sufficiently sticky that other segments can stabilize the rear part of the expanding body as the contractions and expansions move forward. The first instar moves slowly but is quite agile, being able to twist and turn its body to a remarkable degree. Although teased with the hair, the larva did not flex its mandibles, surprising because we assume that it would attempt to defend itself in the case of multiple parasitism (GARM had observed a multiple-parasitized cell with two dead first instars of *Coelioxoides* as well as a live *Coelioxoides* larva). The larva did not open its mandibles widely but rather seemed to nip at the host chorion as it crawled over the surface.

After being teased with the hair, the larva quieted and was next observed with its body and head appressed to the host egg and its

Fig. 23.   First instar of *Coelioxoides waltheriae* with pigmented head capsule clinging to and sucking the yolk of the egg of *Tetrapedia diversipes*. White flocculent material can be seen in the otherwise nearly transparent body of the parasite.

mandibles apparently imbedded into the egg (as in fig. 23). The anterior edge of its enormously broad labrum was pressed against the host chorion and may seal the connection of the parasite's head to the chorion as the liquid yolk of the egg is transferred either by sucking or passive flow into the parasite. No liquid escaped as the larva increased in size and the egg gradually collapsed.

As discussed in the description of the first instar, the enlarged head capsule was initially suspected to be an adaptation permitting a larger array of mandibular muscles to enpower the mandibles, as has been proposed for certain other cleptoparasitic first instars. However, we do not know how the liquid content of the host eggs is transferred to the parasite. A strong pharyngeal pump requiring enlarged muscles might also be an explanation.

Although with some cleptoparasitic taxa, feeding on the host egg (or larvae) is apparently unnecessary, the first instar of *Coelioxoides* grew as it ingested the copious egg content of the giant host egg; in about a 24-hour period, the first instar doubled its length while the body became distended in all directions. No motion of its head capsule or

body surface was detected, but we saw faint motion of internal flocculent material presumably in the gut that was otherwise filled with clear liquid. The first instar continued to feed on the host egg for the remainder of that instar. Partway through the second stage it started feeding on pollen.

We also encountered two other first instars appressed to host eggs, feeding in exactly the same way. This suggests that feeding on the host egg is stereotypic behavior for the species and that the host egg may be nutritionally important for the parasite. If this is true, then it may be necessary for the *Coelioxoides* egg always to be introduced into a cell in which the host embryo is still in the early stage of development, an act that is assured if the parasite female can deposit her egg only through a closure that has not yet hardened. This tentative scenario, of course, needs to be verified with further observations.

All five larvae of *Coelioxoides* watched by GARM (two from Ribeirão Preto and three from São Paulo) underwent three molts suggesting that there are only four larval instars. While most bees have five larval instars as

stated by Trostle and Torchio (1994), this is apparently not universally true.

The final larval instar of *Coelioxoides waltheriae* starts to defecate while it is still feeding, a few hours after the onset of the stadium, as does the final (in that case, the fifth) instar of the host. Fecal pellets differ from those of the host; they tend to be smaller in diameter, and, instead of spindle-shaped (i.e., tapering at both ends), they appear truncated at both ends. Larvae of this species do not spin cocoons. Orientation of the pupae (direction of body relative to cell closure) of *C. waltheriae* in nests seems to be variable, as has been demonstrated for its host.

We have no information as to whether *Coelioxoides waltheriae* spends the cool, dry seasons in diapause as a larva or as an adult.

We did not observe adult *Coelioxoides waltheriae* sleeping near the host nests.

COMPARATIVE DEVELOPMENT RATES OF *TETRAPEDIA* AND *COELIOXOIDES*

Although we were unable to follow the development of *Tetrapedia diversipes* and *Coelioxoides waltheriae* from egg to adulthood, we did monitor their early development. Figure 24 graphically represents the duration of the egg stage and first three or four instars of three individuals of each species based on observations of GARM and IAS. Table 1 provides similar information expressed in hours, which can be compared with the longevity of the stadia of other bee taxa. The data for table 1 are the same used for figure 24 except that their calculation assumes that a change in stage takes place at the midpoint between the last time the earlier stage was observed and the first time the next stage was seen.

The data show that the duration of the egg stage of *Tetrapedia* is longer than 4 days while that of the *Coelioxoides* is less than 1.5 days. The data were gathered under ambient conditions of warm humid days and slightly cooler nights that varied little from one day to the next, so that both developing hosts and parasites were subjected to similar conditions. Hence, the difference in incubation time between host and cleptoparasite is real.

In an attempt to understand the basis for this difference, we reviewed various papers that provided incubation data for other bee species. Hackwell and Stephen (1966) reported that the duration of the egg of *Nomia melanderi* Cockerell was "somewhat longer" than 2.25–3 days. Trostle and Torchio (1994: table 2) stated that the egg stage of *Megachile rotundata* (Fabricius) and *M. apicalis* Spinola lasted 2–5 (average 3.3) and 3–7 (average 4.6) days, respectively. Bosch and Kemp (2000) demonstrated that the duration of the egg stage of *Osmia lignaria* Say was influenced by temperature and sex, with the extreme means ranging from 5.7 to 9.8 days. Rust et al. (1989) reported that eclosion in *Osmia rufa cornigera* (Rossi) occurred 4–4.17 days after egg deposition. Torchio and Burdick (1988) gave the duration of the egg stage for *Epeolus compactus* Cresson and its host *Colletes kincaidii* Cockerell both as 6–9 days. Torchio (1989a) reported that embriogenesis of *Stelis montana* Cresson is completed in 5 days at room temperature. Earlier he (1986) recorded that the embryonic membrane of *Triepeolus dacotensis* (Stevens) ruptured on day 5 to 6 and hatching required an additional 14–18 hours. The incubation period of *Apis mellifera* Linnaeus is generally considered to be 3 days (DuPraw, 1967), as is that of *Scaptotrigona postica* (Latreille) (Beig, 1971). Eggs of *Tetragonisca angustula* (Latreille) take 6 days to hatch (Nates-Parra et al., 1989). H.H.W. Velthuis (personal commun.) thinks that it takes 5 days for eggs of a number of species of *Melipona* to eclose. Thus, the slightly more than 4-day egg stage of *Tetrapedia* appears similar to these taxa, but the less than 1.5-day egg stage of *Coelioxoides* is the shortest by far. We suspect that the need for *Coelioxoides* to acquire the yolk of the host egg may be an explanation for this evolutionary change. Another explanation could be that selection pressure for a shorter egg stage evolved through multiple parasitism, i.e., the first cleptoparasite to hatch presumably has the highest survival value. However, other cleptoparasites, such as the species of *Stelis*, *Epeolus*, and *Triepeolus* cited above, do not have short incubation periods, and multiple parasitism was reported for all three taxa.

An interesting phenomenon reflected in figure 24 and in table 1 is the disparity in the
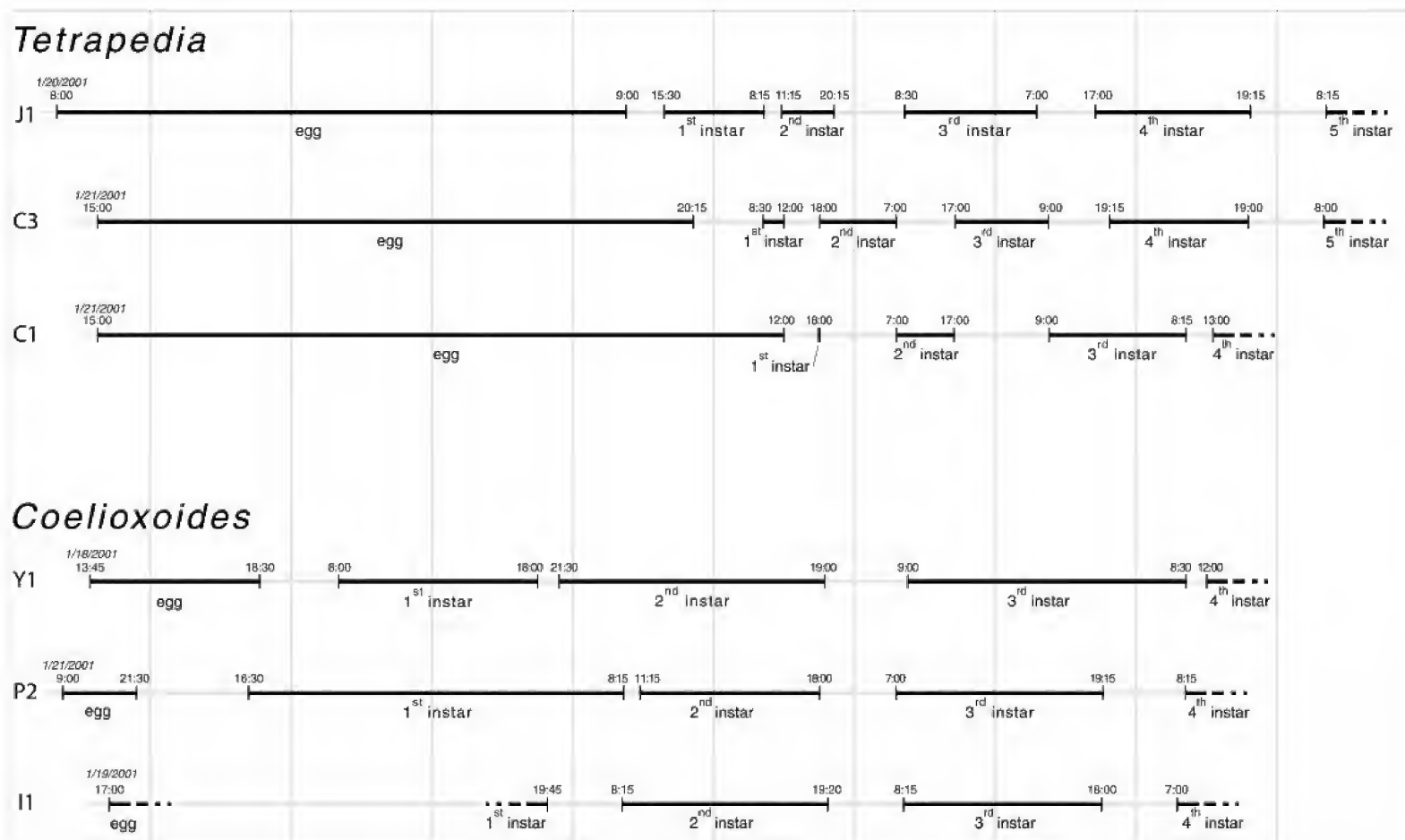
Fig. 24. Duration of egg stage and early larval instars of three individuals each of *Tetrapedia diversipes* and *Coelioxoides waltheriae*, identified by nest code numbers and rounded to closest 15 minutes. Each column represents a 24-hour day starting on the date when the egg was deposited in January 2001. Because it was impossible to continuously monitor development of individuals, gray sections of bars are based on time when the previous stage was last seen and the next stage was first observed. Thus, black sections of bars reflect the known duration of stadia. The exact time of egg deposition is known only for Y1. The long duration of the first instar of P2 is an artifact, created because the specimen accidentally became dislodged from the host egg when they were transported on a bus. After being replaced on the egg, the cleptoparasite did not start feeding again for a day.

TABLE 1
Duration, in Hours, of Egg Stage and Early Instars of *Tetrapedia diversipes* and
Coelioxoides waltheriae
(Data compiled in January 2001. For explanation, see text.)

	Tetrapedia			*Coelioxoides*		
	N	Mean	Range	N	Mean	Range
Egg	3	110	101–120	2	29	23–36
1st instar	3	15	10–22	1	43	—
2nd instar	3	21	17–25	3	47	39–54
3rd instar	3	31	26–34	3	51	47–57
4th instar	2	37	35–38	0	—	—

duration of the early larval instars of *Tetrapedia* and *Coelioxoides*. The first three instars of *Coelioxoides* together take more than twice as long (141 hours) to develop as do the first three instars of *Tetrapedia* (67 hours). Since both host and parasite consume all of the stored food in the cell, this difference in the duration of the feeding instars may simply be a reflection of our observation that the host has five instars and the parasite has four. Since the first instar of neither host nor parasite feeds on provisions, the three following instars of the parasite must consume as much food as the four subsequent instars of the host. Unfortunately, we do not have data on the duration of the last larval instar of either species.

It is possible to compare the average duration of the first three larval instars of these bees with a few others. Hackwell and Stephen (1966: table 1) recorded data on a 12-hour basis on the development of eight specimens of *Nomia melanderi*. We assumed that the time of eclosion was the midpoint between the last time the egg was seen and the first time the first instar was observed. Further, we assumed that the time of molt to the fourth instar was the midpoint between the last time the third instar was seen and the first time the fourth instar was observed. On this basis, the mean duration for these three instars was 75 hours (range 60–96). Trostle and Torchio (1994: table 2), using a much larger sample, found that this period for *Megachile rotundata* averaged 61.6 hours and for *M. apicalis*, 45.6 hours. The mean duration for the same period for *Osmia rufa cornigera* (Rust et al., 1989) was 65.95 hours. Thus, the figure of 67 hours for *Tetrapedia*

roughly groups with these taxa, and the 141-hour period for *Coelioxoides* is remarkably long.

Despite this disparity in the duration of the first three larval instars of *Tetrapedia* and *Coelioxoides*, the total duration from egg deposition to completion of the third instar of the host and parasite is nearly the same. Total mean time for *Tetrapedia* was 177 hours, and for *Coelioxoides* was 170 hours

OOCYTE/OVARIOLE DATA FOR *TETRAPEDIA* AND *COELIOXOIDES*

Table 2 provides statistics regarding the ovarioles, eggs, and mature oocytes of *Tetrapedia diversipes* and *Coelioxoides waltheriae*. The data presented were gathered using the criteria developed by Iwata (1955) and Iwata and Sakagami (1966) and more recently modified by Alexander and Rozen (1987) so as not to distinguish Iwata's category A and B oocytes. The intertegular distance is the maximum distance between the outer rims of the tegulae, measured to the closest 0.1 mm. Because there were no mature oocytes in the seven females of *T. diversipes* whose ovaries we examined, the egg/oocyte length for that species was based on eggs found in nests, also measured to the closest 0.1 mm. The egg/oocyte length of *C. waltheriae* is based on the largest mature oocyte in each of three individuals, similarly measured. The egg index is a measure of egg/oocyte size (length) relative to overall body size (maximum distance between outer margins of tegulae) as proposed by Iwata and Sakagami (1966), as calculated from the

TABLE 2
Ovariole and Oocyte Statistics for
Tetrapdeia diversipes **and** *Coelioxoides waltheriae*
(Numbers in parentheses refer to number of specimens on which values are based. For explanation of terms, see text.)

Taxon	Mean intertegular distance	Mean egg/oocyte length	Egg index	Mean no. mature oocytes per individual	Mean no. mature oocytes per ovariole	Ovarian formula
T. diversipes	2.8 (6)	3.5 (9)	1.25	0 (7)	0 (7)	4:4 (7)
C. waltheriae	2.5 (4)	1.7 (4)	0.68	2 (3)	0.25 (3)	4:4 (4)

mean values in the previous two columns in table 2.

The ovarian formula is the number of ovarioles in each of the two ovaries. As shown in table 2, both host and parasite invariably had 4 ovarioles per ovary, the plesiomorphic number for the Apidae.

The statistics in table 2 for *Tetrapedia diversipes* are noteworthy for two reasons. First, the value 1.25 for the egg index categorizes the egg as a ''giant'' according to Iwata and Sakagmi (1966: table 2). These same authors calculated the egg index of *Tetrapedia maura* to be 1.16 (also a ''giant'') presumably based on an egg length for this species presented by Michener and Lange (1958). The adaptive significance of egg gigantism is explored below in the Discussion on the Eggs of *Tetrapedia* and *Coelioxoides*.

The other striking statistic in table 2 is that none of the seven females whose ovaries we examined contained a single mature oocyte. Indeed, the largest oocyte recovered from one of them was 2.6 mm long (contrasting with a 3.6-mm-long egg that she had deposited). This immature oocyte was more than 1.5 times longer than the entire length of the other ovarioles; hence, its ovariole projected forward in the body cavity far beyond the other ovarioles. Her other oocytes were small and undeveloped. We suspect that the lack of mature oocytes in these females relates to the fact that giant eggs take a long time to develop in females, and this in turn accounts for the slow rate of nest cell construction and provisioning.

The ovariole formula (4:4) of *Coelioxoides waltheriae* contrasts with the largest lineage of cleptoparasitic bees, the Nomadinae, which tend to have an increased number of

ovarioles. However, it does reflect the condition found in five other cleptoparasitic apid lineages (Melectini, Ericrocidini, Protepeolini, Isepeolini, Osirini, and *Exaerete*), which retain the plesiomorphic condition (the one known exception being *Ericrocis lata* [Cresson]) (Alexander and Rozen, 1987; Rozen and Roig-Alsina, 1991; Rozen, 1992, 1994a; Roig-Alsina and Rozen, 1994; Alexander, 1996; and Garófalo and Rozen, 2001). Missing still are ovariole counts for the other parasitic apid lineages (Rhathymini and *Aglae*).

Although based on few specimens, the total number of mature oocytes in *Coelioxoides waltheriae* and the number of mature oocytes per ovariole for this species (table 2) are unusually low for a parasitic bee (Alexander and Rozen, 1987: table 2). We think that this probably relates to slow rate of production of provisioned cells by the host bee; there is no need for this cleptoparasite to have a large number of oocytes to deposit in a short interval because the chances of finding numerous recently completed host cells is low.

The egg index of 0.68 for *Coelioxoides waltheriae* indicates that the oocyte size is large relative to body size for the cleptoparasitic bees dealt with by Alexander and Rozen (1987: fig. 1) and Alexander (1996: table 1). However, the index is low compared with egg indices of solitary bees. This oocyte is described further in the section on immature stages of the species.

DESCRIPTIONS OF IMMATURE STAGES

Roig-Alsina (1990) concluded that *Coelioxoides* was related to its host, *Tetrapedia*, based on four strong synapomorphies of

adults and was supported by a number of other shared adult characters less clearly synapomorphic. An important purpose of the current study is to examine the immature stages (egg, first instar, mature larva, and pupa) of *Tetrapedia diversipes* and *Coelioxoides waltheriae* to shed additional light on the phylogenetic relationships of the two genera. Two descriptions (one of host, the other of parasite) of each stage are presented below. A discussion following each of the paired descriptions explores the significance of the findings.

Because of the conflicting hypotheses through the years regarding the origins of cleptoparasitism among apids, and because *Coelioxoides* has been aligned with other cleptoparasitic apids in the past (see references in Roig-Alsina, 1990, and Alexander, 1996), the discussion following the descriptions also probes alternative affiliations.

The last section of this paper summarizes the relationship of *Tetrapedia* and *Coelioxoides* based on all life stages.

Because eggs and oocytes became somewhat misshapen in preparation for examination by SEM, line illustrations (fig. 25) are presented to show more accurately their unaltered shapes. The descriptions below of eggs/oocytes follow the format used by Rozen (2001b) in describing eggs and oocytes of other bees.

EGG OF *TETRAPEDIA DIVERSIPES*
Figures 25, 26

Egg (figs. 25, 26) large relative to distance between outer rims of tegulae (i.e., egg index 1.25); length 3.4–3.6 mm (N = 9); maximum diameter 1.0–1.2 mm (N = 5). Shape (fig. 25) approximately symmetrical along its strongly curved long axis, robust, rounded at both ends with anterior end only slightly more rounded than posterior end, widest posterior to midlength. Color white. Chorion clear, transparent, smooth, and shiny throughout, without sculpturing or ornamentation even under SEM examination; micropyle not identified.

MATERIAL STUDIED: One egg, IBUSP, São Paulo, Brazil, XII-20–1999 (I. Alves-dos-Santos); 1 egg, same except I-28–2000; 1 egg, Ribeirão Preto, São Paulo, Brazil, IX-

Fig. 25. Diagrams of the eggs of *Tetrapedia diversipes* (right) and *Coelioxoides waltheriae* (left) showing relative sizes and general shape, anterior ends toward the top; chorionic nodules of *Coelioxoides* not depicted. Scale line = 1.0 mm.

9–2000 (G.A.R. Melo, J.G. Rozen); 6 eggs, IBUSP, São Paulo, Brazil, I-15–21–2001 (I. Alves-dos-Santos, G.A.R. Melo, J.G. Rozen).

EGG OF *COELIOXOIDES WALTHERIAE*
Figures 25–31

Egg (mature oocyte) (figs. 25–27) moderately small relative to distance between outer rims of tegulae (i.e., egg index 0.68); length 1.5–1.8 mm (N = 3); maximum diameter 0.38 mm (N = 1). Shape (figs. 25, 27) mostly symmetrical along its slightly curved long axis but apparently slightly flattened laterally at extreme posterior end; oocyte elongate, broadly rounded and widest at anterior end, gradually, evenly tapering to narrowly rounded posterior end; posterior end with dorsal hook-shaped projection but in other cases apparently merely narrowly rounded.

Fig. 26. Live eggs of *Tetrapedia diversipes* (lower left) and *Coelioxoides waltheriae* (upper right) in a freshly opened nest.
Fig. 27. Close-up of the live egg of *Coelioxoides waltheriae* showing nodules covering most of chorion.

Color nearly white. Chorion clear, beset with small, evenly spaced nodules (figs. 28–31; not diagramed in fig. 25) over most of its surface; nodules along outcurve surface each about as high as its basal diameter; nodules smaller along incurve side of egg and gradually becoming absent at anterior end. Under SEM examination: chorion of outcurve surface (figs. 30, 31) finely pitted, faintly divided into irregular polygons by slightly raised ridges, each polygon with single nodule; nodule with irregular granular surface (fig. 31); viewed internally, chorion shallowly dimpled beneath each nodule; chorion at anterior end when viewed externally (fig. 29) with incised, elongate polygons that lack nodules and become increasingly more elongate toward anterior pole; when viewed internally also with incised polygonal structure; micropyle not identified but presumably at anterior pole.

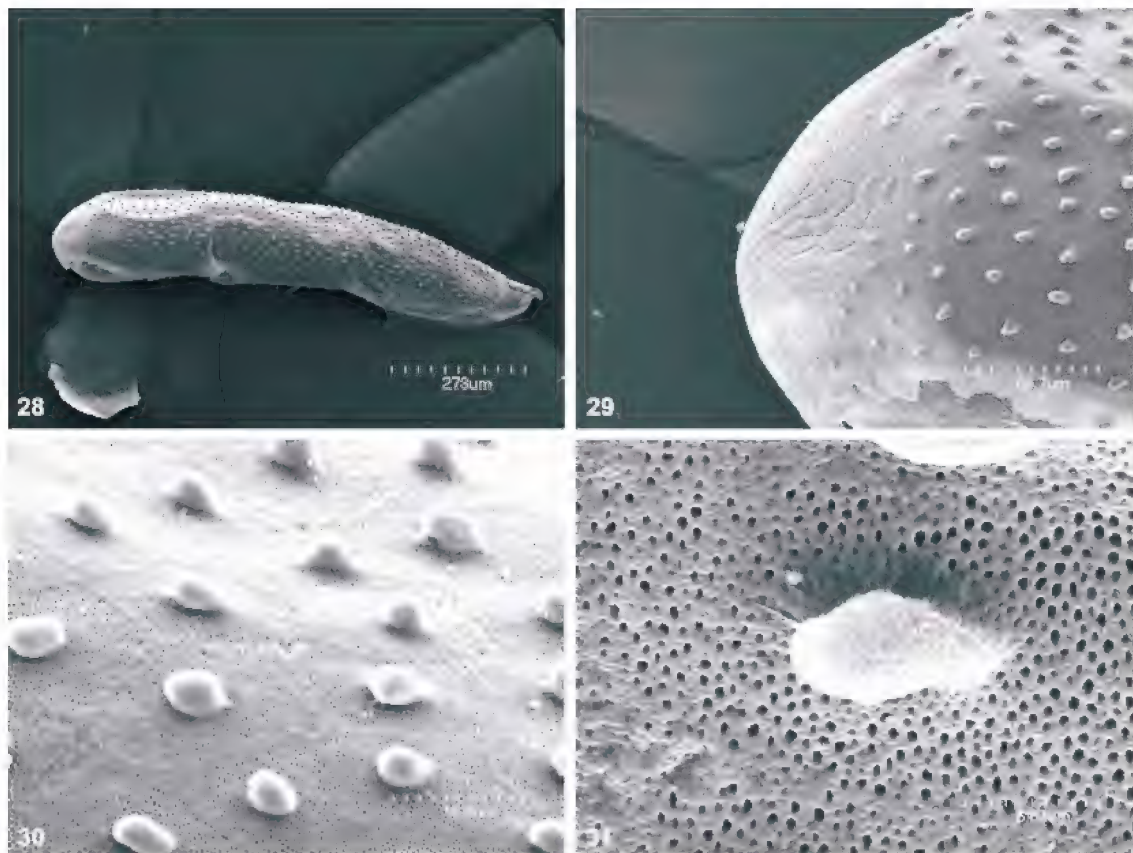MATERIAL STUDIED: One mature oocyte, Campus da USP, Ribeirão Preto, São Paulo, Brazil, IX-13–1998 (G.A.R. Melo); 1 egg, IBUSP, São Paulo, Brazil, XII-20–1999 (I. Alves-dos-Santos); 2 oocytes, same except I-15–21–2001 (I. Alves-dos-Santos, G.A.R. Melo, J.G. Rozen).

REMARKS: In addition to the specimens listed above, we had a chance to observe several live eggs when we met at IBUSP (I-15–21–2001). The hooklike posterior end of the egg seems variable; on one specimen it was sharply pointed and on another it was apparently absent.

The unusual, evenly spaced nodules on much of the surface of the chorion *Coelioxoides waltheriae* is suggestive of a similar array on the oocytes of the cleptoparasite *Epeoloides coecutiens* (Fabricius) (Apinae: Osirini) (Rozen, 2001b).

DISCUSSION OF EGGS OF *TETRAPEDIA* AND *COELIOXOIDES*

Except for its very large size, the egg of *Tetrapedia diversipes* is typical of that of

Figs. 28–31.   SEM micrographs of egg of *Coelioxoides waltheriae*. **28.** Entire egg, lateral view, anterior end to the left. **29.** Close-up of anterior end showing changes in sculpturing at anterior pole. **30.** Close-up of nodules and other surface sculpturing midbody. **31.** Extreme close-up of nodule showing its texture.

most solitary bees. If one accepts the hypothesis of Iwata and Sakagami (1966) that "giant" eggs (egg index >1.10) have been derived from intermediate-sized eggs, then what selection pressure induced this condition? These authors considered a number of factors that might influence increasing egg size and concluded that there is a possible correlation between "subsocial"[5] species and enlarged eggs, as evidenced in the Halictidae and more so in the Xylocopinae. However, *Tetrapedia diversipes* and *T. maura* are solitary. They also considered that characteristics of nesting sites might have given rise to large eggs; large eggs (and

large first instars eclosed from such eggs) would be more resistant to desiccation than would small eggs because of decreased surface/mass ratio. However, they dismissed this possibility, in part because of lack of supporting data, and in part because brood cells are filled with provisions having high water content.

Nonetheless, correlative data lend support to this idea. The following taxa with giant eggs (egg indices in parentheses) nest in wood or stems (all records except for first one from Iwata and Sakagami, 1966): *Tetrapedia diversipes* (1.25), *T. maura* (1.16),[6]

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[5] Iwata and Sakagami (1966) used the term "subsocial" in a different sense than did Michener (1974).

[6] Iwata and Sakagami (1966: table 1) incorrectly coded the nesting habits of this species as being subterranean and burrowing, rather than wood nesting and "renting" (i.e., using preformed cavities).

Hylaeus perforatus Smith, *Xylocopa* 3 spp. (1.38–2.00), *Braunsapis sauteriella* (Cockerell) (1.29), *Ceratina* 7 spp. (1.21–1.80). Michener (1973) addressed egg size in allodapine bees by comparing egg length to body length. Although his index differed from that used here and by Iwata and Sakagami, most of the species had enlarged eggs. Major exceptions, however, were *Allodapula* and *Exoneurella*, which would probably be categorized as small under the Iwata/Sakagami system. No other bees are known to have giant eggs except for eight species of Halictidae. (We do note, however, that some species of Megachilidae nest in wood, and none has giant eggs, so that some explanation will have to be proposed for how they avoid desiccation.)

We also note that provisions with high water content may not surround eggs of *Tetrapedia* since we did not see females adding nectar to the stored food, as is the case with most bees.

At first we thought the egg index of 0.68 of *Coelioxoides waltheriae*, the "small" category of Iwata and Sakagami (1966), argued against the hypothesis that egg gigantism might be adaptive for nesting in wood. After all, the egg of this cleptoparasite occupies the same environment as the host egg. However, two features seem to circumvent the possibility of desiccation of young *Coelioxoides*. First, their eggs hatch quickly (in less than 2 days after oviposition as reported above). Second, the larva immediately affixes itself to the host egg and, during all of its first instar and part of the second, ingests the entire liquid yolk of the host egg, thereby increasing in size dramatically. It is often reported that first instars of cleptoparasitic bees eat the host egg or young larva, and the detailed evidence reported by Linsley and MacSwain (1955) supports such conclusions for *Nomada*. However, JGR has often wondered if such feeding by a cleptoparasite is a nutritional requirement for the parasite or if the host egg or young larva may be consumed accidentally along with the provisions. Such seemed to be the case in the study of *Leiopodus singularis* (Linsley and Michener) (Rozen et al., 1978) where the older host instars, too large to be consumed,

are killed and left to decay in the cells.[7] However, there is no doubt that the first instar and part of the second of *Coelioxoides waltheriae* consume the contents of the host egg, and we suspect that the yolk from the host egg provides both nourishment and water, thus compensating for the small size and limited water content of the small parasite egg.

Not only is the egg of *Coelioxoides waltheriae* much smaller than that of its host, its shape and chorionic morphology are radically different. There are no features of their eggs that suggest a sister relationship of *Coelioxoides* and *Tetrapedia*. Eggs of many cleptoparasitic bees have unusual shapes and chorionic ornamentation contrasting with the stereotypic curved, sausage-shaped eggs of noncleptoparasitic bees. Rozen (1994b) and Rozen et al. (1997) have interpreted egg modifications of cleptoparasites as adaptations whereby the egg is hidden from host females that return to the parasitized cells. However, the present study has determined that the *Coelioxoides* female oviposits by inserting her egg into the cell lumen through the completed cell closure. If this is universally true, then the differences in shape and chorionic structure between the eggs of the two taxa are unexplained.[8]

FIRST INSTAR OF *TETRAPEDIA DIVERSIPES*
Figures 13, 32

DIAGNOSIS: The unpigmented, nonsclerotized head capsule of the first instar of *Tetrapedia diversipes* immediately distinguishes it from the pigmented, sclerotized head capsule of *Coelioxoides waltheriae*. Its man-

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[7] Even in the case of *Leiopodus singularis* there is a question concerning whether it feeds on the host larva. Rozen et al. (1978) observed no indication that the first instar fed on the host, but noted that the first instar increased in size without ingesting whole pollen grains. They tentatively concluded that the pollen grains may have been split apart and the contents ingested, but direct observations are absent.

[8] The chorion of *Coelioxoides* and that of the nonrelated cleptoparasite *Epeoloides* (Apinae: Osirini) are mostly covered with evenly spaced, raised nodules, unusual ornamentation for any bee (Rozen, 2001b). The host of *Epeoloides* is *Macropis*, which, like *Tetrapedia*, provisions its nest with floral oil as well as pollen (Cane et al., 1983); this correlation may or may not be coincidental.
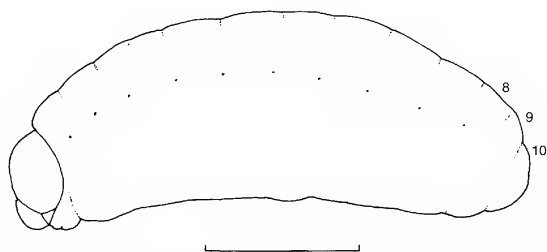
Fig. 32. First instar of *Tetrapedia diversipes*, lateral view, with covering chorion removed. Scale line = 1.0 mm.

dibles are apically bidentate, contrasting with the simple, tapering, apically pointed mandibles of *C. waltheriae*. The labral size of *T. diversipes* is normal, contrasting with the uniquely broad labrum of *C. waltheriae*.

The following description is incomplete, based on a first instar that, though fully developed, was still encased in the chorion. When cleared in a solution of sodium hydroxide, the cranium collapsed, and the postcephalic region folded in on, and adhered to, itself. Hence, many details are missing, but comparison of this first instar with the cast exuviae of a first instar confirmed that the first instar was indeed fully developed.

LENGTH: About 3.6 mm.

HEAD (fig. 32): Hypognathous; parietals normal, not elongate, not enlarged so that foramen normally large compared with head width. Integument of head capsule unsclerotized, unpigmented. Head capsule with sensilla minute, scarcely visible. Condition of tentorium unknown, but thin unsclerotized anterior arms clearly present. Internal head ridges exceedingly thin to absent. Antennae not discernible. Labrum normal in size, about as broad as distance between anterior mandibular articulations, apically weakly bilobed, without tubercles; labral sclerite not evident.

Mandible (fig. 13) apically bidentate with ventral tooth somewhat more robust and slightly longer than dorsal tooth; at least abductor apodeme clearly present. Maxillae present as distinct lobes; palpi and other features not evident. Labium discernible as distinct lobe; palpi, salivary opening not observed.

BODY: Form robust (fig. 32); intersegmental lines weakly incised; intrasegmental lines

not evident; abdominal dorsal tubercles and lateral body swelling absent; prothorax and abdominal segment 9 not protruding ventrally; abdominal segment 10 short, rounded in lateral view. Integument without setae or spicules, but with linear row of granules (not shown in fig. 32) extending between most or all spiracles on each side of body (also evident on cast exuviae). All spiracles present, apparently not projecting beyond body wall. Anus apical.

MATERIAL STUDIED: One first instar (in chorion), 1 cast exuviae of first instar IBUSP, São Paulo, Brazil I-28–2000 (I. Alves-dos-Santos).
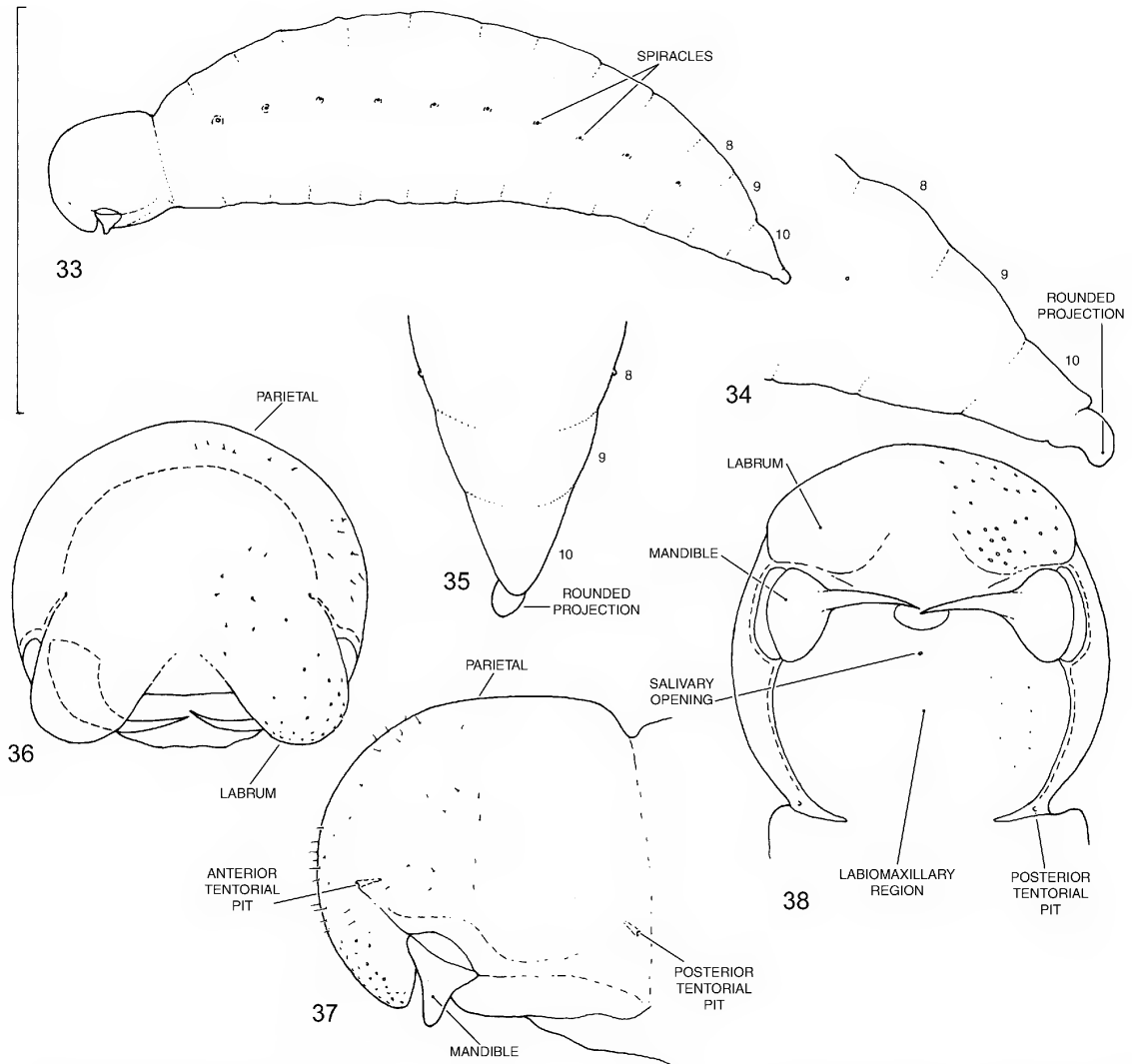
FIRST INSTAR OF *COELIOXOIDES WALTHERIAE*
Figures 33–43

The format of this description is modified from that used by Rozen (1991) for first instars of other cleptoparasitic bees.

DIAGNOSIS: The conspicuous characteristics distinguishing this first stage larva from that of *Tetrapedia diversipes*, exemplar of its presumed closest related genus, are presented in the diagnosis of the latter.

LENGTH: About 1.2 mm.

HEAD (figs. 36–38, 40, 41): Hypognathous; parietals swollen, somewhat elongate in lateral view (figs. 37, 40), somewhat constricted behind so that foramen considerably narrower than maximum head width (figs. 36, 37, 39, 40); posterior part of parietals curving downward and inward but extreme posteroventral ends not meeting ventrally (fig. 38) so that postoccipital bridge incomplete. Integument of head capsule moderately strongly sclerotized, moderately pigmented; integument immediately mesad of anterior part of hypostomal ridge perhaps slightly sclerotized, becoming membranous well before midline. Head capsule and labrum with numerous conspicuous sensilla and without spinulae (as defined for Melectini); head sensilla setiform, those of labral apex nonsetiform. Tentorium incomplete; anterior pits small, arms thin, tapering; posterior pits small, arms thin, perhaps forming bridge; posterior pit near rear of parietal in line with anterior part of hypostomal ridge but above posteroventral end of parietal as seen in lateral view (fig. 37); internal head ridges tend-

Figs. 33–38. First instar of *Coelioxoides waltheriae*. **33.** Entire larva, lateral view. **34, 35.** Last three abdominal segments, lateral and dorsal views, respectively. **36–38.** Head, frontal, lateral, and ventral views, respectively. Scale line (= 1.0 mm) refers to fig. 33.

ing to be obscure apparently because of heavy sclerotization of parietals; sclerotization of parietals ending at postoccipital ridge, which is inconspicuous; anterior part of hypostomal ridge evident, posterior part fusing with and becoming indistinguishable from parietal; integument near ridge not pebbled, wrinkled, or in other ways sculptured; pleurostomal ridge and lateral parts of epistomal ridge moderately developed; epistomal ridge between anterior tentorial pits absent. Parietal bands absent. Antennae absent except per-

haps for several sensilla on each side (figs. 36, 37, 40, 41). Labrum extremely broad, about as broad as swollen head capsule, remarkably bilobed with median emargination separating lobes (figs. 36, 38, 41); entire lower surface of labrum, but especially lobes, beset with numerous nonsetiform sensilla; labral tubercles absent; boundary between labrum and lower end of clypeus indistinguishable, but clypeus very broad and presumably short.

Mandibles (figs. 20, 38) apically attenuate,

curved, sharply pointed, basally moderately broad, without tubercles on outer aspect; inner edge smooth, without teeth or projections. Maxillae, labium, and hypopharynx nonsclerotized, greatly fused, not represented anteroventrally as separate lobes; cardo, stipes, hypopharyngeal groove, and maxillary and labial palpi not expressed; lateral part of labiomaxillary region with scattered sensilla, much less pronounced than those of labrum; salivary opening (with duct attached) posterior to mouth (fig. 38).

BODY: Fusiform, especially long and tapering posteriorly as seen in both dorsal and lateral views (figs. 33, 39); intersegmental lines weakly incised; abdominal segments not divided into cephalic and caudal annulets; abdominal dorsal tubercles and lateral body swelling absent; neither prothorax nor abdominal segment 9 protruding ventrally; abdominal segment 10 tapering, with single rounded projection. Integument without setae, that of each body segment except for abdominal segment 10 with band of spicules extending below level of spiracles (fig. 39); these spicules evenly spaced, short, sharply pointed, directed posteriorly; ventral spiculation completely absent. All spiracles present, projecting beyond body wall; mesothoracic pair of tubercles (fig. 42) projecting the most, with each succeeding pair projecting less than preceding pair; spiracular tubercles apparently sclerotized, each bearing opening apically; diameter of openings of mesothoracic spiracles largest (fig. 42), that of metathoracic spiracles less than half that of first pair; openings of abdominal spiracles small, subequal (fig. 43). Anus not identified.

MATERIAL STUDIED: One first instar, IBUSP, São Paulo, Brazil, I-28–2000 (I. Alves-dos-Santos); two first instars (presumably killed by yet another larva), Ribeirão Preto, São Paulo, Brazil, II-8–1999 (G.A.R. Melo) from nest 6, cell 2, of *Tetrapedia diversipes*.
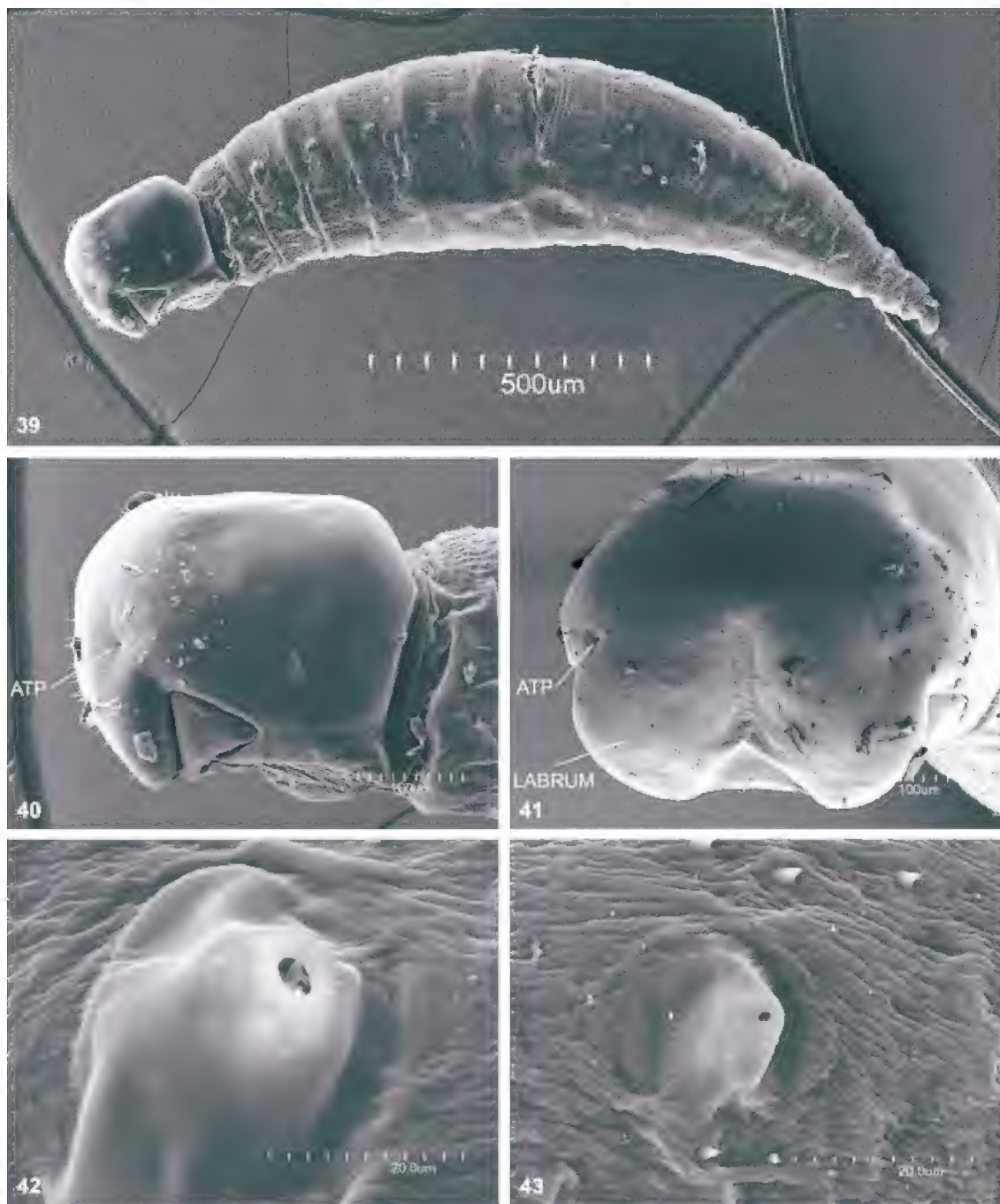
DISCUSSION OF FIRST INSTARS OF *TETRAPEDIA* AND *COELIOXOIDES*

The disparity between the first-instar anatomy of *Tetrapedia diversipes* and *Coelioxoides waltheriae* was predictable. The first instar of *Coelioxoides* is hospicidal, a behavior

that requires the larva to have an internally strengthened head capsule (necessary for increased mandibular power and perhaps for power to suck), elongate, sharply pointed mandibles, modified sensory appendages (for detecting host offspring), and an ability to crawl, all compared with the sedentary, mostly pharate first instar of *Tetrapedia*. There are no certain synapomorphies shared by the two; the few similarities are presumably plesiomorphic. Hence, comparing these two larvae does not inform us about the phylogenetic relationships of the two taxa.

Does the anatomy of the first instar of *Coelioxoides* suggest a relationship of that taxon with any other cleptoparasitic lineage? Rozen (1991) presented a comparative study of the cleptoparasitic Anthophoridae as revealed by their mode of parasitism and the anatomy of the first instars. Six other cleptoparasitic groups were treated: Nomadinae, Protepeolini, Melectini, Ericrocidini, Rhathymini, and Isepeolini. The tentative conclusion was that cleptoparasitism had a separate evolutionary origin in each of these taxa, with the possible exception of the Isepeolini and Ericrocidini. Missing from the study were first instars of *Coelioxoides* and the Osirini. Subsequent to that investigation, Roig-Alsina and Michener (1993) concluded that the Anthophoridae were paraphyletic and placed the included taxa with the corbiculate apids (including the cleptoparasitic *Exaerete* and *Aglae*) into the Apidae. First instars of the Osirini and *Aglae* have yet to be studied. Garófalo and Rozen (2001), after studying the larval instars and mode of cleptoparasitism of *Exaerete smaragdina* (Guérin), concluded that its first instar is pharate within the chorion and incapable of killing host eggs. However, its second instar, with sharply point, curved mandibles, was capable of killing immatures of host and other cleptoparasites, although the cleptoparasitic female may kill the host eggs when she oviposits.

Using the 21 characters identified by Rozen (1991: table 1), the following are the character states in boldface coded for *Coelioxoides* (0 indicates plesiomorphic state): (0) Introduction of cleptoparasite egg into host cell: **2—through closure after cell is closed.** (1) Egg deposition: **3—free in cell, in food**

Figs. 39–43. SEM micrographs of first instar of *Coelioxoides waltheriae*. **39.** Entire larva, lateral view. **40.** Head, lateral view, with anterior tentorial pit (ATP) identified. **41.** Head, anterofrontal view, with anterior tentorial pit (ATP) and labrum identified; note absence of antennae. **42.** Spiracular tubercle of prothorax. **43.** Same of abdominal segment 8; note reduced size of atrial opening.

TABLE 3
Derived Character states of Egg Deposition Features and First Instars
Shared by *Coelioxoides* with Other Cleptoparastic Apidae
(For further explanation, see text.)

Taxa	Shared derived states	No. shared states
Coelioxoides/Nomadinae	11, 14, 16	3
Coelioxoides/Protepeolini	11	1
Coelioxoides/Melectini	0, 12, 14	3
Coelioxoides/Rhathymini	0, 3, 11, 14	4
Coelioxoides/Isepeolini	11, 12, 16	3
Coelioxoides/Ericrocidini	0, 11, 12, 14, 16	5

mass. 2. Head shape: **0—hypognathous.** (3) Parietal: **2—swollen, globose.** (4) Ventral sclerotized postoccipital bridge: **3—partly present, not fused.**[9] (5) Head sclerotization: **0—ending at posterior margin.** (6) Cranial band of spinulae: **0—absent.** (7) Troughlike external hypostomal groove: **0—absent.** (8) Angle of posterior margin of head to hypostomal groove: **0—right angle.** (9) Antennal size: **3—not evident.** (10) Antennal shape: **?** [antenna not evident]. 11. Antennal fusion: **1—presumably fused with head capsule.** (12) Labrum: **2—sclerotized [at least at base] with clypeus.** (13) Labral tubercles: **4—absent.**[10] **14. Mandible: 1—moderately long.** (15) Labiomaxillary sclerotization: **0—absent.** (16) Labium and maxilla: **1—extensively fused.** (17) Maxillary palpus: **4—absent.**[11] (18) Abdominal segment 10: **3—tapering, apically nearly pointed.** (19) Body setae: **0—absent.** (20) Spiracles of abdominal segment 8: **0—normal in position.**

Table 3 indicates the derived character states of egg deposition features and first instars shared by *Coelioxoides* with other cleptoparasitic apids (excluding *Exaerete*) whose first instars are known. The states for *Coelioxoides* are those listed in the precedi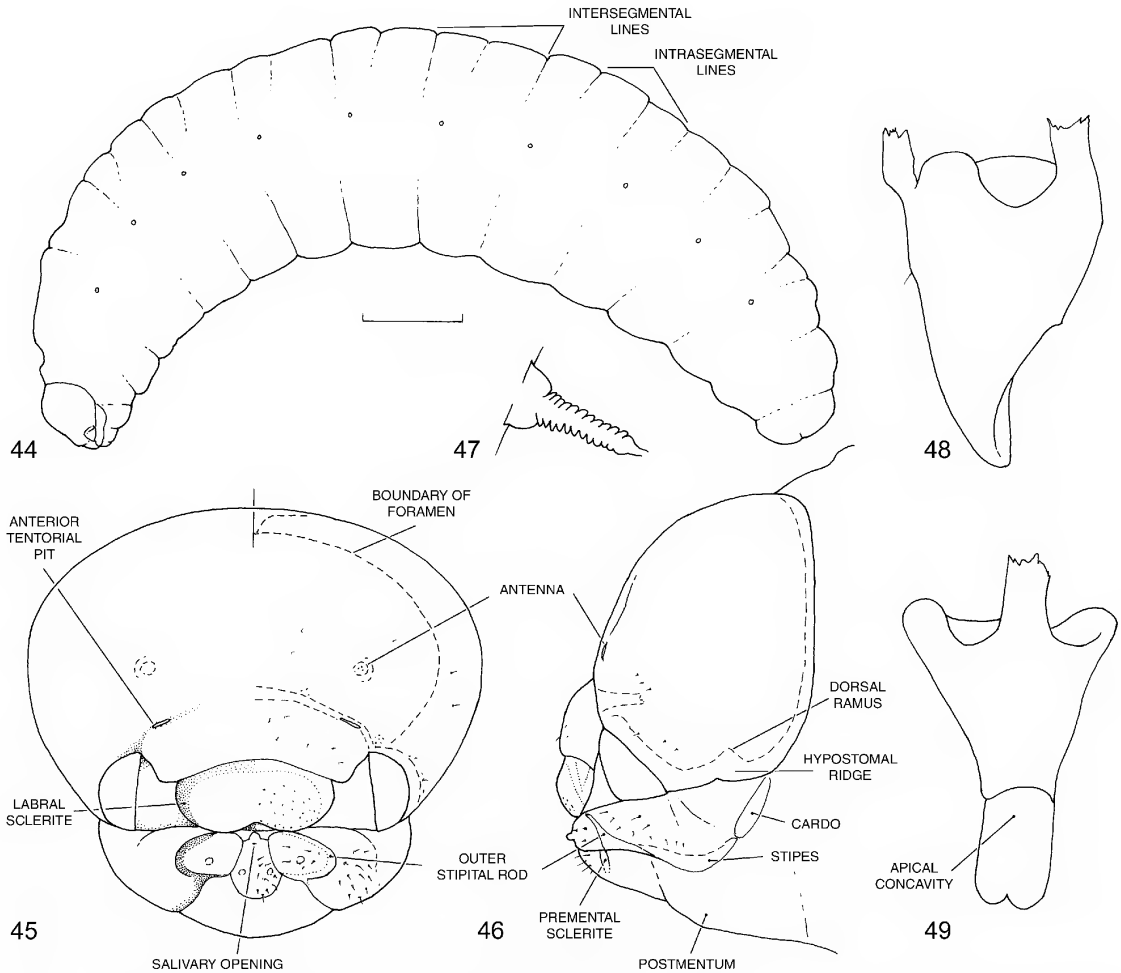ng paragraph; the states for other cleptoparasitic apids come from Rozen (1991: table 2). Hence, table 3 is a continuation of table 3 presented by Rozen (ibid.). By comparing these two tables, one sees that *Coelioxoides* shares relatively few features with the other cleptoparasitic apid lineages and confirms that there is no reason to conclude that *Coelioxoides* shared a common cleptoparasitic ancestor with any of the other lineages. This is further emphasized by the fact that six derived character states (characters 1, 4, 9, 13, 17, 18) possessed by *Coelioxoides* (as coded above) are unique to that genus. This is the greatest number of autapomorphies possessed by any of the lineages (number of autapomorphies of other lineages: Nomadinae, 1; Protepeolini, 4; Melectini, 1; Rhathymini, 1; Isepeolini, 4; Ericrocidini, 0). Other characters presented in the description but not treated by Rozen (1991) further emphasize the distinctiveness of *Coelioxoides*.

Of all known first instars of cleptoparasitic apids, only *Coelioxoides* and *Rhathymus* have head capsules that are swollen and therefore strikingly similar. Greatly expanded parietals (permitting attachments of enlarged mandibular muscles to operate the essential killing structures, and possibly, in *Coelioxoides*, musculature to allow it to suck the content of the host egg) is unknown elsewhere in the Apidae (although present in some cleptoparasitic Megachilidae). Since Rozen (1991: table 1) hypothesized that prognathism arose independently in four different lineages as a special adaptation for host assassination, a de novo origin of cranial swelling, presumed adaptation for the same function (increasing musculature power to deal with host offspring), is equally likely.

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[9] Alternatively, this state might have been coded 1 as a transition step toward a complete bridge.

[10] The expanded lateral lobes of the clypeus are not termed tubercles because they are not considered homologs of those of the Nomadinae, as evidenced by the dissimilar labra of mature larvae of these two taxa.

[11] The palpus may be represented by sensilla, but, since the sensilla present cannot be distinguished from one another either in the first or last larval instar, the maxilla is coded as absent.

Figs. 44–49.   Postdefecating larva of *Tetrapedia diversipes*. **44.** Entire larva, lateral view. **45, 46.** Head, frontal and lateral views, respectively. **47.** Spiracle, side view. **48, 49.** Right mandible, dorsal and inner views, respectively. Scale line (= 1.0 mm) refers to fig. 44.

MATURE LARVA OF *TETRAPEDIA DIVERSIPES*
Figures 17–19, 44–49

DIAGNOSIS: Features that are unusual or unique and which therefore may aid in identification are in boldface below. Important characters shared with *Coelioxoides waltheriae* that might serve to identify larvae of the Tetrapediini are presented in italics in the description of *C. waltheriae*.

DESCRIPTION: Length (if straight) approximately 9.5 mm.

Head (figs. 45, 46): **Integument unpigmented except for following areas:** hypostomal ridges, anterior mandibular articulations, **labral sclerite, paired lateral sclerites of epipharyngeal surface (presumably the tormae),** mandibles, cardines, stipites including articulating arms, and **lateral parts of premental sclerite;** cranium with scattered, often setiform sensilla; clypeus with conspicuous, often setiform sensilla; labrum with numerous conspicuous nonsetiform sensilla, most abundant apically; prementum and apices of maxillae with conspicuous setiform sensilla that are longer than those elsewhere on head; head without distinct spicules although base of postmentum with granular surface similar to patches of integument immediately behind head.

Head size moderately small compared with body; head capsule wider than long in frontal view. Tentorium complete, robust except dorsal arms scarcely developed; **anterior tentorial pit elongate,** slightly closer to anterior mandibular articulation than to antenna; posterior tentorial pit well impressed, found at junction of hypostomal ridge and postoccipital ridge. Coronal ridge developed only on vertex. **Postoccipital ridge** well developed, its **median portion curving forward;** hypostomal ridge well developed; dorsal ramus of ridge incomplete; pleurostomal ridge moderately developed; **epistomal ridge well developed its entire length. Parietal band not evident. Antennal prominence not developed;** antennal disc differentiated from papilla; antennal papilla scarcely projecting, with approximately three sensilla. Front of head capsule as seen in lateral view (fig. 46) sloping normally. **Labrum broad as seen in frontal view, with distinct basal sclerite that curves along lateral margins as characteristic of Megachilidae and *Bombus*; labral apex emarginate medially; labral disc and apex without paired tubercles; epipharynx** a simple, slightly curved surface, **laterally with pair of elongate sclerites (tormae) that apically meet distal ends of labral sclerite.**
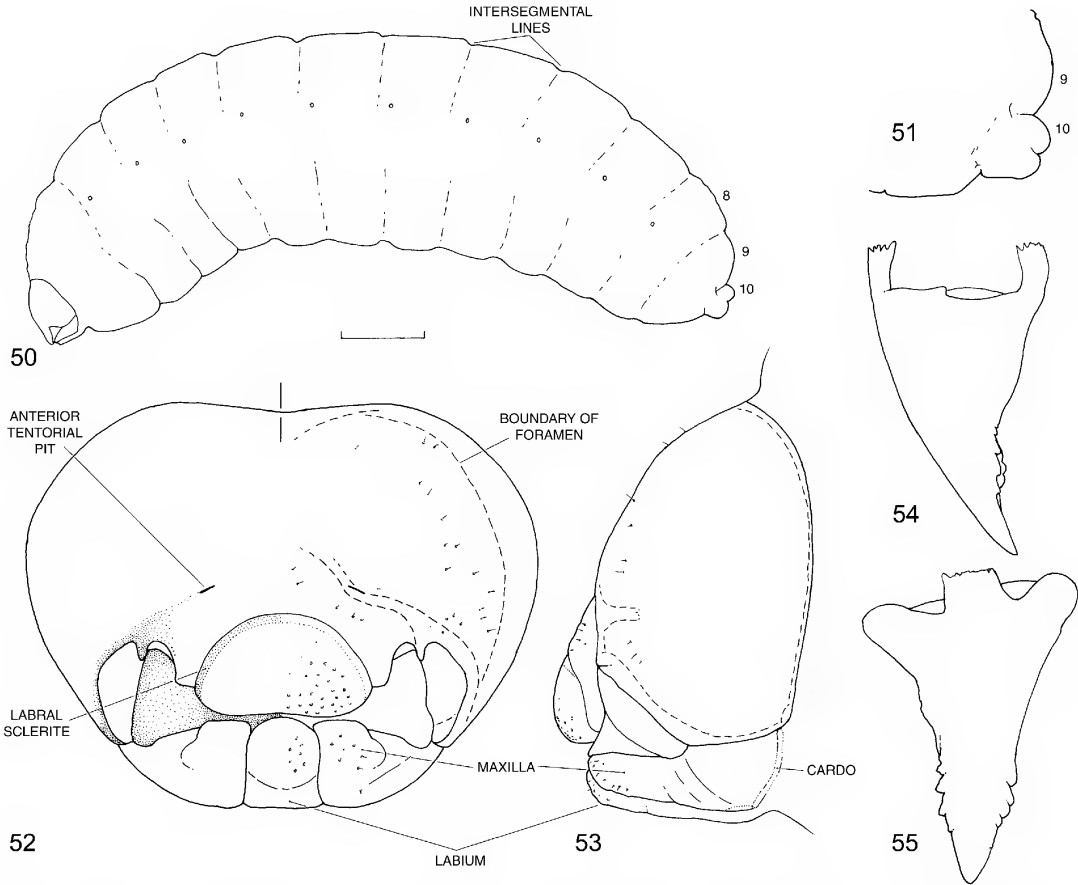
Mandible (figs. 17–19, 48, 49) robust, moderately long; outer surface without tubercles but with one or two short setae; **mandibular apex bidentate; apical teeth of predefecating larva apparently sharply pointed with ventral tooth longer and stouter than dorsal tooth; apical teeth of postdefecating larva nearly identical as seen in adoral view (fig. 49), now broadly rounded apically presumably because of wear; apical concavity large, smooth, scoop-shaped; upper and lower apical edges without denticles or teeth.** Labiomaxillary region (fig. 46) not strongly projecting but also not greatly recessed. Maxillary apex well separated from labium, produced mesally, and bearing small palpus that is about as long as its basal diameter; galea not evident; cardo and stipes well developed; **anterior end of stipes dividing ventrally into outer rod that curves around outer surface of maxillary apex and inner rod that curves adorally to cibarium;** stipital articulating arm arising from inner rod; faintly pigmented area extending forward from inner arm along inner surface of maxillary apex; articulating arm of stipes elongate. Labium weakly divided into prementum and postmentum; **premental sclerite conspicuous as two lateral pigmented sclerites; prementum small with narrowly rounded apex bearing salivary opening;** labial palpus small, nearly identical in size to maxillary palpus. Salivary opening small, simple, without projecting lips; opening positioned at upper apical edge of labium with dorsal surface of labium directed posteriorly immediately behind it; hypopharyngeal groove not evident. Hypopharynx without lobes, a simple continuation of dorsum of labrum to buccal cavity.

Body (fig. 44): Integument without setae except for minute setiform sensilla on abdominal segment 10; integument very finely, transversely wrinkled, without spicules although finely irregular in areas just behind head; integument without spines or sclerotized tubercles. **Body form slender, linear;** intersegmental lines moderately defined; dorsal intrasegmental lines evident on most body segments; **dorsal, paired dorsal, and lateral body tubercles completely absent;** abdominal segment 10 in lateral view (fig. 44) attached medially to segment 9; anus apically positioned on segment 10 as seen in lateral view; perianal area without ridges. **Spiracles (figs. 47) extremely small,** subequal in size; atrium projecting beyond body wall, with rim; peritreme moderately wide; primary spiracular opening with collar; subatrium moderate in length, with approximately 10 chambers; atrial and subatrial walls without denticles or other ornamentation. Male with cuticular scar centered on venter of abdominal segment 9; female sex characters unknown.

MATERIAL STUDIED: One male postdefecating larva, Ribeirão Preto, São Paulo, Brazil, II-22–1999 (G.A.R. Melo), nest 6, cell 1.

REMARKS: Although the description of this instar was based on the material listed, we examined numerous other last instars when we met at the University of São Paulo, São Paulo, January 15–21, 2001.

Figs. 50–55. Postdefecating larva of *Coelioxoides waltheriae*. **50.** Entire larva, lateral view. **51.** Apex of abdomen enlarged, lateral view. **52, 53.** Head, frontal and lateral views, respectively. **54, 55.** Right mandible, dorsal and inner views, respectively. Scale line (= 1.0 mm) refers to fig. 50.

MATURE LARVAE OF *COELIOXOIDES*
WALTHERIAE
Figures 22, 50–55

DIAGNOSIS: The very small abdominal segment 10 of the larva of this species is unique in bees and will, therefore, permit immediate identification. Absence of maxillary and labial palpi and of antennal disc and papilla are also unique for the larvae of any long-tongue bees, although palpi are greatly reduced in some xylocopines. Characters that distinguish the mature larva of this species from that of *Tetrapedia* are given in boldface, and larval characters that *Coelioxoides* and *Tetrapedia* share and that therefore might be tribal are in italics.

The larva of this species has been included in a key to the mature larvae of cleptoparasitic bees (Rozen, 2001a).

DESCRIPTION: Length (if straight) approximately 9.0 mm.

Head (figs. 52, 53): *Integument unpigmented except for following areas: hypostomal and pleurostomal ridges, epistomal ridge laterad of anterior tentorial pits, labral sclerite, paired lateral sclerites of epipharyngeal surface (presumably the tormae),* mandibles, *cardines, stipites, antennae, and lateral parts of premental sclerite;* cranium with scattered setiform sensilla; clypeus with conspicuous setiform sensilla; labrum with numerous conspicuous nonsetiform sensilla, most abundant apically; prementum and apices of maxillae with conspicuous sensilla, those at apices nonsetiform and those sub-

apical areas setiform, of about same length as those on cranium; head without spicules.

Head size moderately small compared with body (fig. 50); head capsule much wider than long in frontal view (fig. 52). Tentorium incomplete because larva preparing for ecdysis, and apodemes incomplete; anterior tentorial pit elongate; **posterior tentorial pit scarcely impressed,** found at junction of hypostomal ridge and postoccipital ridge. Median longitudinal thickening of head capsule absent. *Postoccipital ridge* moderately robust on vertex where *its median portion curves forward;* this ridge becoming narrower laterally; **hypostomal ridge** moderately well developed but **without any evidence of dorsal ramus;** pleurostomal ridge moderately developed; epistomal ridge well developed laterad of anterior tentorial pits; **mesad of pits, ridge arching dorsally and then fading almost completely.** *Parietal bands not evident. Antennal prominence not developed*; **antennal disc, papilla, and apparently sensilla absent.** Front of head capsule as seen in lateral view (fig. 53) sloping normally. *Labrum broad as seen in frontal view, with* distinct semicircular *basal sclerite;* labral apex almost straight, only faintly emarginate medially; *labral disc and apex without paired tubercles;* **epipharynx** a simple surface, laterally **with pair of short sclerites (tormae)** apically attached to distal ends of labral sclerite.

Mandible (figs. 22, 54, 55) long, tapering to simple, acute apex; projecting cusp and apical concavity absent; upper and lower apical edges coarsely, irregularly serrate; outer surface without tubercles and apparently without setae or sensilla. *Labiomaxillary region (fig. 53) not strongly projecting but also not greatly recessed.* **Maxillary apex** well separated from labium, directed mesally, **without palpus** although some sensilla may be vestiges of palpus; galea not evident; *cardo well developed; stipes well developed* as a narrow rod basally; where inner surface of maxilla attaches to labium, **stipes branching so that inner rod continues to curve toward cibarium and outer rod bends abruptly at right angle and extends over top of maxilla where it ends;** articulating arm of stipes not discernible but perhaps present. Labium not clearly divided into

prementum and postmentum, poorly developed so that, in lateral view of head (fig. 53), labium greatly eclipsed by maxilla; premental sclerite expressed as vague pigmented areas on sides of labium; **labial palpus absent** although some sensilla may be vestiges. *Salivary opening small, simple, without projecting lips; opening positioned just behind upper apex of labium with dorsal surface of labium directed posteriorly immediately behind it; hypopharyngeal groove not evident. Hypopharynx a continuation of dorsum of labium to buccal cavity, without lobes.*

Body (fig. 50): Integument without setae except for minute setiform sensilla on abdominal segment X; *integument without spicules, spines, or sclerotized tubercles. Body form* moderately slender, *linear;* intersegmental lines moderately defined; **dorsal intrasegmental lines absent;** *dorsal, paired dorsal, and lateral body tubercles completely absent;* **abdominal segment 10 very small relative to segment 9, about one-half diameter of 9 in lateral view (fig. 50),** attached medially to segment 9; *anus apically positioned on abdominal segment 10;* perianal area without ridges. *Spiracles small, subequal in size; atrium projecting beyond body wall, with rim;* peritreme of moderate width; *primary spiracular opening with collar; subatrium moderate in length,* with 7–15 chambers; atrial and subatrial walls without denticles or other ornamentation. Male sex characters unknown; female with paired cuticular scars ventrally on abdominal segments 7–9.

MATERIAL STUDIED: One female postdefecating larva, Ribeirão Preto, São Paulo, Brazil, III-11–1999 (G.A.R. Melo), nest 4.

REMARKS: The description of this instar was based on the material listed. In addition, we examined other specimens when we met at the University of São Paulo, São Paulo, January 15–21, 2001.

DISCUSSION OF MATURE LARVAE OF *TETRAPEDIA* AND *COELIOXOIDES*

Characters presented in italics in the description of *Coelioxoides waltheriae* above support the inclusion in the Tetrapediini if they are considered synapomorphies. The following is an attempt to evaluate the

strength and weakness of these features. (1) Because pigmentation and sclerotization of the cardo, stipes, and premental sclerite are found in some other taxa but not usually in taxa that do not spin cocoons, this may be a strong synapomorphy. (2) Forward curving of the postoccipital ridge on the vertex has been reported by McGinley (1981: cladistic character 17) to occur in a number of unrelated bee taxa, including some in the Apidae, and is less supportive than point 1, above. (3) Complete absence of parietal bands and antennal prominences are unusual and seem to be strong. (4) The absence of the antennal disc and papilla in *C. waltheriae* appears to contrast with the presence of these structures in *Tetrapedia diversipes*. However, these structures are weakly developed in the later, an unusual feature for bee larvae, so that the condition of the antennae in these two species may represent steps in a transformation series from the normal, well-developed antennae of other bee larvae. (5) The shared labral features are of uncertain value in that they are known in *Ctenoplectra*,[12] *Bombus*, and a few Melitomini of the Apidae and they are similar to those of the Megachilidae. (6) Another transformation series may be the shared, more-or-less weakly developed labiomaxillary region that extends forward almost like that of a cocoon-spinning larva

[12] At the time that JGR described the larva of *Ctenoplectra* (Rozen, 1978), he was unaware of the possible significance of the labral sclerite and therefore did not interpret the labral morphology correctly. Reexamination of the specimen shows that a distinct, faintly pigmented labral sclerite is present, arching across the base of the labrum. The apical bilobed condition of the labrum is an expression of the median apical emargination as found in the Tetrapediini. Unlike in the Tetrapediini, the labrum of *Ctenoplectra* is normal in width, that is, not especially broad. Michener and Greenberg (1980) pointed out a number of larval characters shared by *Ctenoplectra* and Megachilidae (including the Fideliinae): paired labral tubercles absent; apical mandibular concavity strong and scooplike; and apex of maxilla produced mesally. The presence of a distinct labral sclerite is one more character shared by these taxa. *Tetrapedia* possesses all of these features. In addition, larval *Ctenoplectra* and *Tetrapedia* share a well-developed median section of the epistomal ridge and a linear body form lacking dorsal tubercles. Larval *Ctenoplectra* differs by possessing elongate palpi and antennal papillae, a pronounced, dorsally projecting hypopharynx, and distinct, projecting salivary lips, and by lacking an outer sclerotized rod at the stipital apex.

(even though the labium is more reduced in *C. waltheriae* than in *T. diversipes*). (7) The position of the salivary opening at the upper apical edge of the labium with the dorsal surface of the labium directed posteriorly immediately behind it is unusual and therefore may be an important synapomorphy. Most bee larvae bear the salivary opening more centrally on the apex of the labium. (8) The shared non-developed hypopharynx is a part of this character complex and adds weight to the possibility that these features are a synapomorphy. Less convincing are the smooth body integument, lack of dorsal tubercles, linear body form, and centrally positioned abdominal segment 10 since these features are associated with other wood- and stem-nesters such as *Hylaeus* and the Xylocopinae.

Character states presented in boldface in the description of the mature larva of *Coelioxoides waltheriae* contrast sharply with the states of corresponding characters of *Tetrapedia diversipes*. In addition to these obvious differences, the head capsule of *T. diversipes* is constricted behind near the posterior tentorial pits so that the foramen is small relative to the head capsule outline (fig. 42), and the front-to-back length of the head capsule is normal. Such features occur in most bee larvae. In contrast, the head capsule of *C. waltheriae* is not so constricted behind near the posterior tentorial pits (fig. 53) (hence the shallowly impressed posterior tentorial pits), and the front-to-back length is shorter.

The shared characters presented above may not be convincing when taken separately. However, in aggregate they do seem to support the sister group relationship of *Tetrapedia* and *Coelioxoides*. The numerous dissimilarities of the larvae of these two taxa may have been imposed by the development of a parasitic way of life in *Coelioxoides*.

A comparison of the mature larva of *Coelioxoides* with those of other cleptoparasitic apids whose larvae have been collected is possible because representatives of most tribes have been described (see McGinley, 1989, and Rozen, 2001a, for references). Rozen (1996) presented a listing of 13 synapomorphies of mature larvae of the Nomadinae. He considered eight of these to be especially strong. The following comparisons of features of *Coelioxoides* with these eight

(numbered as in Rozen, 1996) are as follows: (1) Unlike in the Nomadinae, labrum of *Coelioxoides* completely lacking acute tubercles arising from disk. (2) Unlike in Nomadinae, labiomaxillary region not markedly recessed and not greatly fused; maxillae and labium quite distinct. (3) As in Nomadinae, labium not divided into prementum and postmentum. (4) Unlike in Nomadinae, cardo, stipes, and possibly articulating arm of stipes present. (5) As in Nomadinae, labial palpus not expressed by more than sensilla, but, unlike in the Nomadinae, maxillary palpus also not expressed by more than sensilla. (8) Unlike in Nomadinae, internal head ridges moderately expressed. (10) As in Nomadinae, mandible apically simple, without projecting cusp or scooplike apical concavity, but this is considered a convergence to a parasitic way of life because of the tapering, sharply pointed mandible of the hospicidal first instar. Furthermore, unlike in most Nomadinae, the mandible of the last instar is moderately elongate and not short like those of most nomadines. (12) As in the Nomadinae, the parietals with their posteroventral edge (in vicinity of posterior tentorial pits) not strongly inflexed so that foramen magnum is not constricted at level of posterior tentorial pits, but, unlike in the Nomadinae, the parietals are expanded. This contradiction in the description of this feature suggests a different underlying explanation of its function and therefore a separate origin. In conclusion, the few similarites between *Coelioxoides* and the nomadines do not support the idea of a common cleptoparasitic ancestor.

Comparisons of the mature larva of *Coelioxoides* with those of the cleptoparasitic Apinae whose larvae are known (Protepeolini, Isepeolini, Melectini, Rhathymini, and Ericrocidini) reveal no derived similarities.

PUPA OF *TETRAPEDIA DIVERSIPES*
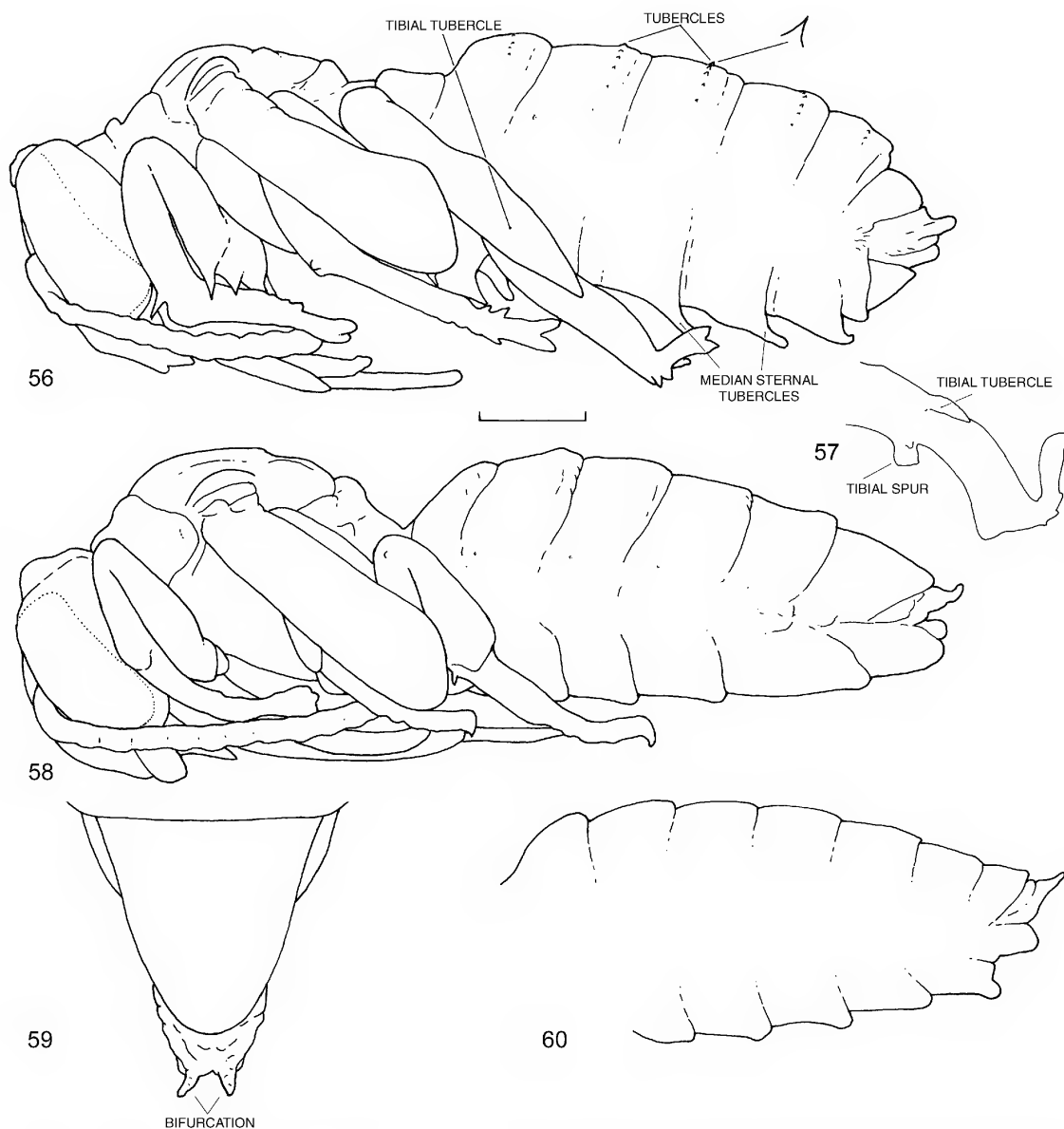Figures 56, 57

DIAGNOSIS: Although pupae of the Apinae are poorly known, the lack of both mesoscutal and mesoscutellar tubercles apparently will serve to distinguish the pupa of this species from those of the following, which have paired, either mesoscutal and/or mesoscutellar tubercles: Isepeolini (Rozen, 2000), Osi-

rini (ibid.), Protepeolini (Roig-Alsina and Rozen, 1994), Exomalopsini (Rozen and Michener, 1988), Tapinotaspidini (Rozen and Michener, 1988), Emphorini (Michener, 1954), Eucerini (Mohamed, 1974), Anthophorini (Michener, 1954), Centridini (Camargo et al., 1975; Rozen and Buchmann, 1990), Rhathymini (Camargo et al., 1975), Ericrocidini (Rozen and Buchmann, 1990; Rozen, 2000), Melectini (Rozen, ibid.), Euglossini (Zucchi et al., 1969). The presence of the long, tapering careotubercle (defined in Methods and Terminology) on the apex of the foretibia of the pupal *Tetrapedia diversipes* will distinguish this species from known pupae of the Meliponini (Michener, 1954; Lucas de Oliveira, 1968, 1970), Bombini (Michener, 1954), Apini (Michener, 1954), and perhaps all other apine tribes. Pupae of the Ancylini and Ctenoplectrini are unknown.

Features that are unusual or unique and that therefore may aid in identification are in boldface below.

HEAD: Integument without setae, spicules, tubercles, or verrucae. Apex of clypeus without rounded, downward-projecting swelling on each side of labrum as in Ammobatini; **labrum about as long as maximum width, its apex pointed in frontal view;** pupal ocelli scarcely defined, nontuberculate. **Mandible with subapical rounded ventral tubercle accommodating developing setae.**

MESOSOMA: **Integument without setae or verrucae.** Lateral angles and posterior lobes of pronotum scarcely produced, corresponding to those of adult. Mesepisternum without tubercles; mesoscutum and mesoscutellum without tubercles, not produced; metanotum not produced. Tegula not produced, without tubercle(s); wings without tubercles. **All coxae and trochanters with long, tapering, sharply pointed, ventroapical tubercles that accommodate developing setae; forefemur with sharply pointed, ventrobasal tubercle; midfemur of female with similar tubercle but that of male apically rounded; hind femur of female with small, more or less evident, rounded, ventrobasal tubercle, that of male without tubercle, possibly because of swollen femur; foretibia with elongate (length about four times basal diameter), tapering, sharply pointed**

Figs. 56, 57.   Pupa of *Tetrapedia diversipes*. **56.** Entire body of female, lateral view. **57.** Apex of male hind leg, lateral view.

Figs. 58–60.   Pupa of *Coelioxoides waltheriae*. **58.** Entire body of female, lateral view. **59.** Apex of female metasoma, dorsal view. **60.** Metasoma of male, lateral view. Scale line (= 1.0 mm) refers to figs. 56–58, 60.

careotubercle (see definition in Methods and Terminology) arising from mesoanterior apex; midtibia with small, apically rounded tubercle on outer surface; hind tibia of female with very large apical tubercle on outer surface; this tubercle in male smaller than that of female; this tubercle in both sexes appressed to basitarsus; hind tibial spur simple, moderately large in female, in male spur a large bulbous swelling anteriorly bearing small tubercle at base (fig. 57); tarsomeres with small but sharply defined ventral tubercles.

METASOMA: Integument without spicules

(except for very fine ones on intersegmental membrane) or setae; **T1–5 (female), T1–6 (male) each with subapical row of very small, sharply pointed tubercles;** sterna without rows of sharply pointed tubercles but **S1–5 of male each apically with single, median, acutely rounded, small tubercle and S1–5 of female each with apical, tapering, elongate, apically blunt, median tubercle, much larger than that of male,** these tubercles, at least in female, accommodating developing adult setae. Apex of metasoma without terminal spine, ending as a rounded membranous lobe as seen from above.

MATERIAL STUDIED: Two female pupae, Campus do USP, Ribeirão Preto, São Paulo, Brazil, IX-16–1998 (G.A.R. Melo), from nest in carton tube; 1 male pupa, São Paulo, São Paulo, Brazil, nest D8 (I. Alves-dos-Santos); 1 female pupa, same except I-27–2000.

REMARKS: Other pupae were examined at the University of São Paulo, São Paulo, January 15–21, 2001.

PUPA OF *COELIOXOIDES WALTHERIAE*
Figures 58–60

DIAGNOSIS: The complete absence of transverse rows of sharply pointed tubercles on all pupal terga is unknown for any other taxon in the Apidae. Lack of mesoscutal and mesoscutellar tubercles is an uncommon feature in the family, although this character is held in common with *Tetrapedia*.

Characters that distinguish the pupa of this species from that of *Tetrapedia* are given in boldface, and pupal characters that *Coelioxoides* and *Tetrapedia* share and that therefore might be tribal in nature are in italics.

Rozen (2000) presented a key that can be used to distinguish this pupa from pupae of other cleptoparasitic bees.

HEAD: *Integument without setae, spicules, tubercles, or verrucae.* Apex of clypeus without rounded, downward-projecting swelling on each side of labrum as in Ammobatini; labrum longer than maximum width, its apex a simple curve in frontal view; pupal ocelli moderately defined, nontuberculate. **Mandibles without swellings or tubercles.**

MESOSOMA: *Integument without setae, spicules, or verrucae. Lateral angles and posterior lobes of pronotum scarcely produced, corresponding to those of adult.* Mesepisternum without tubercles; *mesoscutum and mesoscutellum without tubercles, not produced; metanotum not produced.* Tegula not produced, without tubercle(s); wings without tubercles. **All coxae, trochanters, femora, and tarsi without tubercles; tibiae without tubercles except for acute apical swelling accommodating outer apical spine of adult female; tibial spurs on hind legs absent.**

METASOMA: Integument without spicules and setae; **terga and sterna without tubercles (including those arranged in subapical transverse bands) or projections. Apex of metasoma** without distinct terminal spine but **with small membranous bifurcation as seen from above (fig. 59),** this bifurcation not containing developing adult structure.

MATERIAL STUDIED: One female pupa, Ribeirão Preto, São Paulo, Brazil, II-08–1999 (G. Melo), *Tetrapedia* nest 7, cell 1; 1 female pupa, São Paulo, São Paulo, Brazil, 1–27–00 (I. Alves-dos-Santos); 1 male pupa, University of São Paulo, São Paulo, January 15–21, 2001 (I. Alves-dos-Santos, G.A.R. Melo, J.G. Rozen).

REMARKS: The maxillary palpus (not visible in fig. 58) is a thumblike appendage on the pupa, considerably more pronounced than the scarcely discernible, scalelike adult palpus.

DISCUSSION OF PUPAE OF *TETRAPEDIA* AND *COELIOXOIDES*

Pupae of *Tetrapedia diversipes* and it cleptoparasite, *Coelioxoides waltheriae*, differ in numerous ways as indicated in boldface in the description of the latter. The absence of leg tubercles in pupal *Coelioxoides* can be explained by its having very short adult hairs in contrast to the numerous tubercles (except for the foretibial careotubercles) of *Tetrapedia*, which has long setae in adult males and females. Similarly, the peculiar median sternal tubercles of female *Tetrapedia* accommodate developing adult setae; these tubercles are absent in *Coelioxoides*. Pupal similarities of these two taxa, that is, lack of tubercles on head and especially mesosoma, however, are uncommon features in the Ap-

idae and therefore may reflect common ancestry. Another, possibly less likely, scenario is that the similarities may reflect evolutionary convergence since bees that nest in wood or stems (*Hylaeus*, *Chilimelissa*, *Ceratina*, *Xylocopa*) perhaps tend to have pupae with less mesosomal ornamentation.

The pupa of *Coelioxoides* bears little resemblance to known pupae of other cleptoparasitic Apidae (see McGinley, 1989, and Rozen, 2000, for references).

CONCLUSIONS REGARDING RELATIONSHIPS OF *TETRAPEDIA* AND *COELIOXOIDES*

Of all immature stages, the mature larvae of *Tetrapedia* and *Coelioxoides* show the greatest number (eight as listed in the discussion section) of character states that can be interpreted as synapomorphies. For pupae, only one synapomorphy has been identified, that is, the lack of tubercles on the head and especially on the mesosoma. First instars exhibit no features that can be considered synapomorphic, but this is not surprising considering the very different role of the hospicidal first-instar *Coelioxoides* from the probably inactive first-instar *Tetrapedia*. The morphology of the eggs/mature oocytes of these taxa share no similarities with each other, presumably because those of *Coelioxoides* are adaptive to a parasitic life. The shared features of the mature larvae and pupa support the hypothesis presented by Roig-Alsina (1990) based on adult characters (i.e., these two genera are sister taxa).

However, immatures of these two genera must also be compared with those of other apids to ascertain if synapomorphies might be detected revealing other relationships. To some extent this has been done. Immatures of *Coelioxoides* have been contrasted with those of other cleptoparasitic apids whose immatures are known. The one universal similarity of first instars of all known cleptoparasitic apids is the elongate, curved, sharply pointed mandibles, necessary for killing host immatures. However, differences in first instars as well as eggs, last larval instars, and pupae abound. As detailed in the discussion sections of this paper, convincing synapomorphies between *Coelioxoides* and the other cleptoparasitic apids (to the extent known) cannot be found, or, if suspected, they are not supported by character states in other life stages. For example, the swollen, hypognathous head of first-instar *Coelioxoides* and *Rhathymus* was a match but was not supported by features of the last larval instars or pupae of the two.[13] This similarity is therefore probably an evolutionary convergence.

The life stages of both *Coelioxoides* and *Tetrapedia* must also be compared with those of the nonparasitic apids, a task that is beyond the scope of this study. Except for the last larval instar, the nonparasitic apids are too poorly known or (as in the case of eggs) are probably too plesiomorphic to be of analytical value at present. The mature larvae of these two genera appear to bear no strong resemblance to those of the Exomalopsini, Tapinotaspidini, Ctenoplectrini, Emphorini, Eucerini, Anthophorini, and Centridini (again, see McGinley, 1989, and Rozen, 2001a, for references), although at some point a critical analysis of the immature stages of the apids should be undertaken.

One is left with two impressions: (1) The immatures of *Coelioxoides* and *Tetrapedia* are quite distinct from those of other known apids. (2) While these two genera are probably sister genera based on the similarities identified by Roig-Alsina (1990) and by this study, they are quite distinct from one another based on features of the eggs, first instars, and pupae, a conclusion paralleling closely the one expressed by Roig-Alsina based on adults.

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[13] Bohart's judgment (1970) that *Rhathymus* and the ericrocidines might have had a common parasitic origin is not supported by the very different first instars of these two taxa (Rozen, 1991). Their mature larvae do show some striking similarities (Rozen, 1969b) in the enlarged labiomaxillary region, body form, and mandibular shape, but, if these are synapomorphies, they are likely derived from a common, nonparasitic ancestor. It is unlikely that a single cleptoparasitic lineage would have evolved two such entirely different first-instar anatomies to assassinate host immatures (Rozen, 1991).

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